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AD613617
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WT-1013
(Supersedes ITR-1063)

Operation WIGWAM

May 1955

Project 2.5

EFFECTS OF NUCLEAR EXPLOSION ON MARINE BIOLOGY

Issuance Date: May 31, 1957

COMMANDER TASK GROUP 7.3

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WT-1013
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Report to the Scientific Director

EFFECTS OF NUCLEAR EXPLOSION ON MARINE BIOLOGY

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THIS REPORT HAS BEEN APPROVED FOR OPEN PUBLICATION.

Scripps Institution of Oceanography
University of California
La Jolla, California
August 1956

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ABSTRACT

In order to provide biological information for the conducting of this weapon test in such a manner as to eliminate or minimize hazard through contamination of commerical fisheries products, investigations were conducted along three lines:

1. Studies of the distribution of marine organisms in and near the proposed test area to provide information which, together with data from Project 2.8 on currents, would make possible the selection of a test location such that the hazard to the fisheries would be minimal.

2. Laboratory studies on the uptake of fission products by fishes and other marine organisms to provide basic information on the uptake of such products from sea water and their retention and excretion by the organisms.

3. Field studies following the test to investigate the uptake of resulting fission products by marine organisms.

On the basis of these studies, together with information from other projects, it was possible to select a test location which resulted in no hazard to commercially important types of fish. However, such events occurring in biologically more productive areas, closer to commercial fishing areas, or in regions where the contaminated water would be carried into commercial fishing areas could result in important hazards. The laboratory and field data reported here should provide some basis of evaluating the hazards involved in the occurrence of such events in other areas.

PREFACE

The investigations reported here were undertaken by personnel of several divisions of the Scripps Institution of Oceanography. Members of the faculty of these divisions gave much valuable advice and assistance to members of the research team. The masters and crews of the vessels Paolina-T, Horizon, and Spencer F. Baird, aboard which the field work was conducted, are to be thanked for their helpful cooperation in the work, especially in regard to the experimental fishing conducted from the first-named vessel.

Background information, most of which had not yet been published, was obtained from the Atomic Energy Commission, especially from its New York Operations Office, personnel of which were also very helpful in providing advice on certain problems of instrumentation and radiological analysis.

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CHAPTER 1

SUMMARY

1.1 OBJECTIVE

The investigations undertaken under this project were of three kinds:

1. Studies of the distribution of marine organisms in and near the proposed test area to provide information which, together with data from Project 2.8 on currents, would make possible the selection of a test site such that the hazard to the fisheries would be minimal.

2. Laboratory studies on the uptake of fission products by fishes and other marine organisms to learn about the uptake of such products from sea water and their retention and excretion by the organisms.

3. Field studies following the test to investigate the uptake of resulting fission products by marine organisms.

1.2 DISTRIBUTION OF MARINE ORGANISMS IN AND NEAR THE TEST AREA

1.2.1 Marine Phytoplankton

Two surveys were conducted, one in May and June 1954 and the other in April 1955, to determine chlorophyll concentration and photosynthetic rates in different parts of the region as indications of the abundance of phytoplankton, which supports the organisms higher in the food chain.

It was shown that, except in the portions of the area immediately adjacent to the coast, the region is low in phytoplankton. The northwestern part of the region of study, in the vicinity of 123°W, 28°N was particularly barren. (Further details of this study are given in Chap. 2.)

1.2.2 Marine Zooplankton

Five surveys were conducted before the test between April 1954 and March 1955, and a further survey was made in the immediate vicinity of the test site following the test in May 1955.

It was found that zooplankton standing crops decreased with distance from shore in all parts of the area and that rather low volumes of zooplankton usually occur in the northwestern sector of the region studied. There was no detectable seasonal change. Volumes of less than 50 ml of zooplankton per 1000 m^3 of water were found at all times offshore from about 122° west longitude in the pretest series, although one sample of 55 ml per 1000 m^3 was obtained in the posttest sampling. (Details of these studies are given in Chap. 3.)

1.2.3 Fish Eggs and Larvae

The commercial fisheries off Baja California are conducted well inshore from the proposed test site. However, some of the commercial fishes (sardines, for example) are known to move offshore to spawn. In order to determine to what extent the commercial species might be present in the different parts of the area for spawning, plankton hauls taken on four cruises during 1954 were examined for fish eggs and larvae of all kinds.

Eggs and larvae of several species of commercial fishes were taken on these cruises, but with the exception of dolphin and jack mackerel, either none or very small numbers were taken beyond about 200 miles offshore. No larvae of tunas, which are the basis of the most important pelagic fishery off Baja California, were captured. It seemed probable, therefore, that selection of a site well offshore would result in little hazard due to the presence of spawning concentrations of commercial fishes. Certain noncommercial groups, especially stomiatoids and myctophids, were found in fair numbers in the offshore sector of the study region. (A detailed report on these studies is given in Chap. 4.)

1.2.4 Deep-sea and Forage Fishes

Midwater trawling was done on one pretest cruise in June 1954, and further midwater trawl hauls were made after the test in May 1955. Fish taken with the midwater trawl were all indigenous to the deep-sea environment and included no commercial species. Numbers captured in the vicinity of the test area were notably lower than for similar hauls in coastal waters, which substantiated the findings from other lines of study of the general paucity of marine life in the offshore region off the northern part of Baja California. (Further details are reported in Chap. 5.)

1.2.5 Tunas and Other Large Pelagic Fishes

The principal pelagic commercial fishery off Baja California is that for the tunas. Tropical tunas, the yellowfin and skipjack, are taken off the southern part of Baja California and at the Revillagigedo Islands through the year. During the summer and fall these species are present farther northward to at least Cedros Island. During the month of May, these tropical tunas are seldom taken north of about Magdalena Bay. Albacore tuna are taken to the north, from the vicinity of Guadalupe Island northward, from July to November or December. Bluefin tuna are taken from Guadalupe Island northward at any time of year but always well inshore.

From the distribution of the commercial catch, it seemed likely that a test site north of about 26° north latitude and offshore well outside Guadalupe Island would present little likelihood of having any appreciable numbers of tuna in May. Because, however, of the lack of commercial fishing offshore and because of the importance of this matter, four survey cruises to fish for tuna and other pelagic fishes by long lines were made from May to October 1954, and still another cruise was made in March and April 1955.

As a result of these cruises, it was concluded that the quantity of tunas and other large commercial fishes in the offshore, northerly part of the region surveyed was very small at any time of year and that it could be expected to be essentially zero in the early spring months.

Since, however, there is a large population of tuna supporting a commercial fishery inshore and south of 25°N during the test period, it was necessary to also be assured that water contaminated with fission would not be transported into this area of the commercial tuna fishery. Oceanographic studies under Operation Wigwam Project 2.8 indicated that it would be necessary to place the test site west of approximately 123° west longitude to accomplish this.

Long-line fishing in the vicinity of the test site, just prior to and after the test, confirmed the absence of significant numbers of tunas or other large pelagic commercial fishes in the area at the time of the test. (Details are reported in Chap. 6.)

The data are summarized in Chap. 7.

1.3 LABORATORY STUDIES OF UPTAKE OF FISSION PRODUCTS BY ORGANISMS

1.3.1 Uptake of Sr^{90} and Y^{90} by Marine Microorganisms

Experiments were conducted on the uptake of these fission products by Serratia marinorubra and Platymonas subcordiformis in radioactive media. Serratia marinorubra cells were shown to be able to concentrate the activity from 6000 to 25,000 times. Of the activity retained, 95 per cent was due to Y^{90}, and 4 per cent was due to Sr^{90}. On the basis of atom uptake, more Sr^{90}, approximately 130 times as much, was found. The percentage values for concentration of atoms showed a greater concentration for Y^{90} than for Sr^{90}, presumably because of the 3592 to 1 ratio of Sr^{90} and Y^{90} in the medium initially. Platymonas subcordiformis cells selectively concentrated Y^{90} more than Sr^{90}. On a percentage-atom-uptake basis, Y^{90} was concentrated to a greater degree than Sr^{90}. (Details of these experiments are given in Chap. 7.)

1.3.2 Consumption of Microorganisms Labeled with Sr^{90} and Y^{90} by the Copepod Tigriopus californicus

Experiments by feeding the above-named microorganisms, which had taken up Sr^{90} and Y^{90}, to this harpacticoid copepod, were conducted to determine the rate of feeding of the copepod and the transfer of activity up this step in the food chain. Results were not conclusive. It is indicated that there was little or no concentration of activity by Tigriopus californicus in the presence of Serratia marinorubra. There is, however, evidence of Tigriopus californicus grazing on Platymonas subcordiformis and thereby accumulating activity from Sr^{90} and Y^{90}. (Details of these experiments are given in Chap. 8.)

1.3.3 Uptake of Fission Products by Phytoplankton

Experiments were conducted between May 20 and 30, 1955, to determine the amount of radioactivity taken up by laboratory cultures of a marine dinoflagellate, Gonyaulax polyedra, from sea water containing mixed fission products collected in the Wigwam test area. A concentration of activity in the cells of this organism of about 5000 times in a period of 90 hr was indicated. From concentration factors in different dilutions of radioactive sea water, it appears that in the range of 5 to 50 per cent dilution, the concentration factor is independent of dilution. Studies of energy spectra of the cells which had taken up activity suggest differential uptake of certain energies. The corresponding isotopes were not identified. (Details are given in Chap. 9.)

1.3.4 Elemental Composition of Some Pelagic Fishes

In order to evaluate the potential uptake of fission products and the potential sites of concentration of various elements, it was of interest to learn something of the concentration of various naturally occurring elements in the various organs of pelagic fishes. This was undertaken by studying the elemental composition of various organs of tunas and other pelagic fishes.

The data indicate that the elements existing most probably as cationic species in sea water (Mn, Cu, Ni, Zn, etc.) tend to be concentrated in the internal organs. The alkaline earths (Ca and Sr) concentrate in the hard parts, and Sr appears more strongly in the flesh than Ca. The abundance of Sr is found to be greatest in the internal organs and least in the hard parts. (Details of the analyses are given in Chap. 10.)

1.3.5 Uptake of Radiostrontium by Pacific Mackerel

An experiment was conducted to determine the uptake, retention, excretion, and sites of deposition of Sr^{90} in a representative pelagic food fish, the Pacific mackerel (Pneumatophorus

diego), by feeding this isotope and studying total activity and its distribution in various organs after various periods of time up to 235 days. It was found that 95 per cent of the activity was excreted in 24 hr and that the remaining 5 per cent remained fixed in the body for the duration of the experiment. Eighty per cent of this fixed activity was located in the skeletal structures. The edible portion of the fish showed, per gram, low activity after two days. After one to three days following the time of feeding, the gills showed the highest activity per unit weight, suggesting this as the site of major excretion. (Details of this experiment are contained in Chap. 11.)

1.4 FIELD STUDIES IMMEDIATELY AFTER TEST TO STUDY UPTAKE OF FISSION PRODUCTS BY MARINE ORGANISMS

Radiochemical studies of sea water, of the particulate matter in the sea water, and of the organisms were made on the basis of samples collected at the test site during the first few days after the shot.

Approximately half the activity in the sea water was found to be due to materials present in particulate form.

The most active organisms during this early time, and hence the most effective concentrators of activity, were mucous, pseudopodal, ciliary-feeding zooplankton species which, it was presumed, were ingesting the particulate matter. Limited assays of diatoms indicated low effectiveness in accumulating activity, although this result is somewhat doubtful owing to the possibility that the technique was faulty.

During this early period the fishes showed no significant concentrations of activity except in the stomach and gut regions, indicating (1) that they were feeding on organisms lower in the food chain which were radioactive and (2) that the active elements had not yet reached deposition sites in the other parts of the fish. No long-term studies for sites of accumulation of specific isotopes were conducted. (Details of this part of the research are given in Chap. 12.)

CHAPTER 2

MARINE PHYTOPLANKTON—AREAL SURVEYS

By R. W. Holmes

Two surveys were carried out, one in May and June 1954 and the other in April 1955, to determine the photosynthetic rates and chlorophyll a concentrations of the phytoplankton at the surface in the pelagic area. Since there is good agreement between the magnitude of photosynthetic rates and chlorophyll a concentrations at the surface and those for the entire euphotic zone, it was believed that surface observations would give reliable indications of the total amount of photosynthesis and plant pigment in the water column at any point.

In general, wherever there exists within an area both low photosynthetic activity and low standing crops, the area in question will be incapable of supporting sizable populations of zooplankton and fish for any appreciable length of time. This would not be true if conditions were such that large populations of zooplankton were being introduced into a barren area by currents. Under such conditions a sizable fish population might be sustained in an area of low photosynthesis and low phytoplankton standing crop.

The results of the 1954 cruises (chlorophyll a only) are illustrated in Fig. 2.1. In this figure it is immediately apparent that the northwestern portion of the pelagic area has the lowest phytoplankton standing crop—chlorophyll a concentrations of less than 0.10 mg/m^3; in fact, these concentrations are among the lowest recorded in the northeastern Pacific Ocean. The tongue of relatively rich water that may be seen (Fig. 2.1) extending almost due westward in the vicinity of 28°N, 115°W was apparently transitory; it had completely vanished by the end of May 1954. The area immediately adjacent to the Baja California coast was quite rich and probably remains so. In April 1955 a cruise into the northwestern part of the pelagic area (stations marked on Fig. 2.1) again revealed this area to be one characterized by low phytoplankton standing crop. The chlorophyll a concentrations at all stations were less than 0.10 mg/m^3 and generally less than 0.08 mg. Taken together, these data certainly indicate the very sparse phytoplankton populations inhabiting the northwestern portion of the pelagic area.

Although the 1954 photosynthetic measurements are suspect, they indicate in a general way very low production rates throughout the area, excepting perhaps the region adjacent to Baja California. Similar measurements, quantitative in this instance, were made in April 1955. These data reveal that very low photosynthetic rates are common in the northwestern portion of the pelagic area.

In conclusion it may be said that the pelagic area, neglecting that portion of the area immediately adjacent to the coast, is very barren of phytoplankton, especially in the northwestern corner of the area, and is incapable of supporting large populations of zooplankton and/or fish.

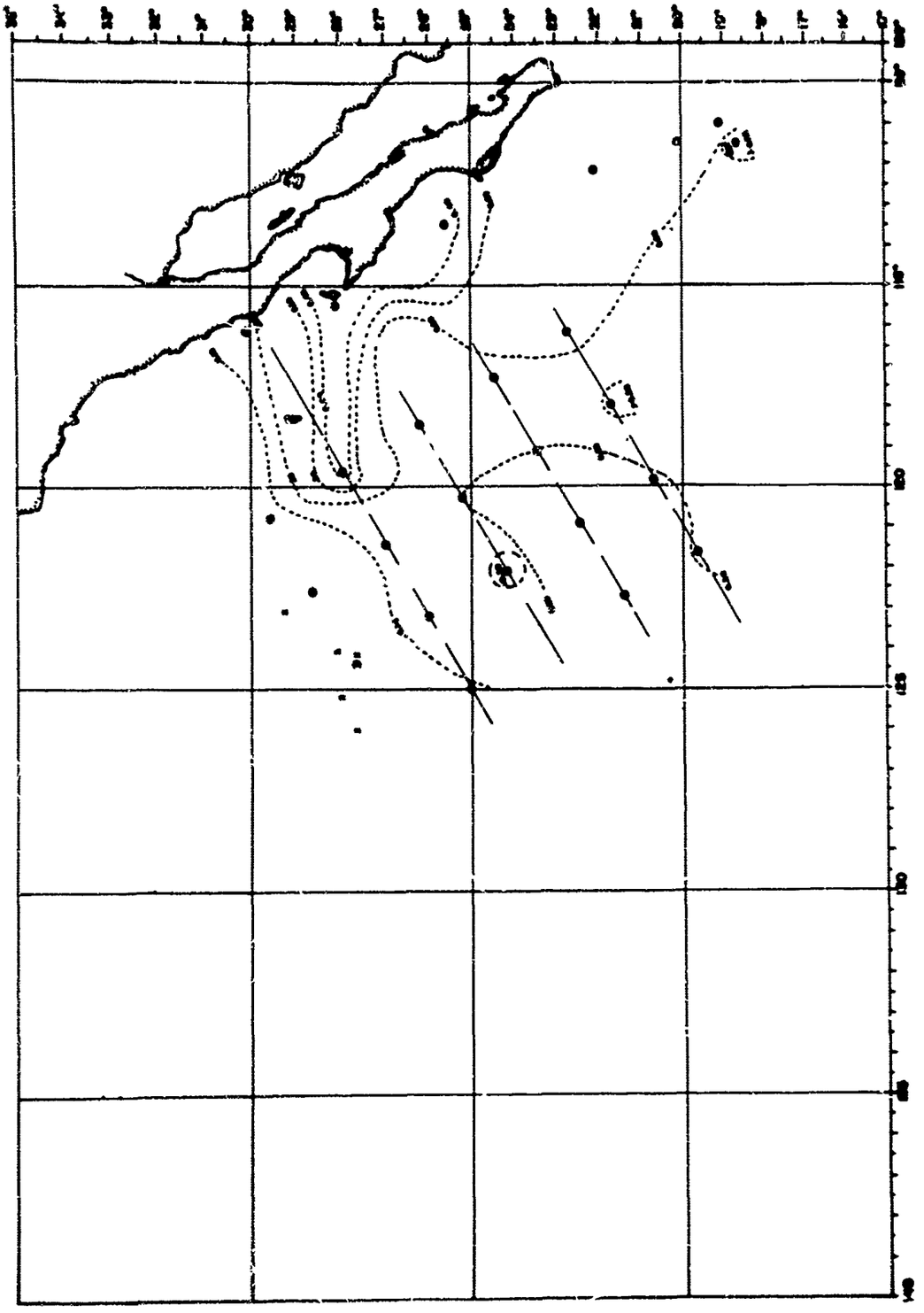

Fig. 2.1—Chlorophyll distribution, May and June 1954. O, stations occupied in May and June 1954. X, stations occupied in April 1955. The values shown are in milligrams per cubic meter.

CHAPTER 3

ZOOPLANKTON INVESTIGATIONS

By Robert Bieri

3.1 INTRODUCTION

Marine zooplankton is the assemblage of animals which are unable to effectively swim against the horizontal currents of the oceans. This assemblage is of direct importance to man in several respects but chiefly because it forms the bulk of the food of most fish and the baleen whales. Recent investigations have shown that they concentrate radioactive substances and are probably instrumental in passing these active elements on to fish.

Previous to the initiation of the pelagic area survey (PAS), our knowledge of the zooplankton in the PAS zone was derived from plankton hauls taken at three Mid-Pacific Expedition stations, seven Shellback Expedition stations, and about 20 California Cooperative Oceanic Fisheries Investigations (CCOFI) stations (Fig. 3.1). These latter were occupied monthly from 1949 through 1954. In addition, numerous plankton tows taken around the area by these expeditions and the CCOFI program gave us some information as to how the area fits into the broader picture of the North Pacific Ocean zooplankton.

Both Shellback and Mid-Pacific indicated that there is a sharp faunal break at about 20° north latitude between what has been called the "Pacific central fauna" and the "Pacific equatorial fauna" (Fig. 3.2). There is a faunal transition, less marked and apparently more variable, passing through the PAS area from northwest to southeast and marking the change from Pacific central fauna to California current fauna.

From these early observations it was known that the highest zooplankton volumes (greatest standing crop) occur in the California current fauna, with rather high values also occurring in the vicinity of the faunal break at 20°N. The lowest zooplankton volumes were known to occur in the Pacific central region.

Because practically nothing was known of the vertical distribution of zooplankton in the PAS region, the early phases of the zooplankton investigations were largely concerned with this problem.

3.2 PRETEST METHODS

Zooplankton varies in size from microscopic single-celled animals a few thousandths of an inch long to huge jellyfish that may reach 10 ft in diameter and 30 ft in length. Because no single device catches all the wide range of sizes, three different nets were used in the PAS work. The largest net (0.7-mm mesh opening) was 1 meter in diameter at the mouth and about

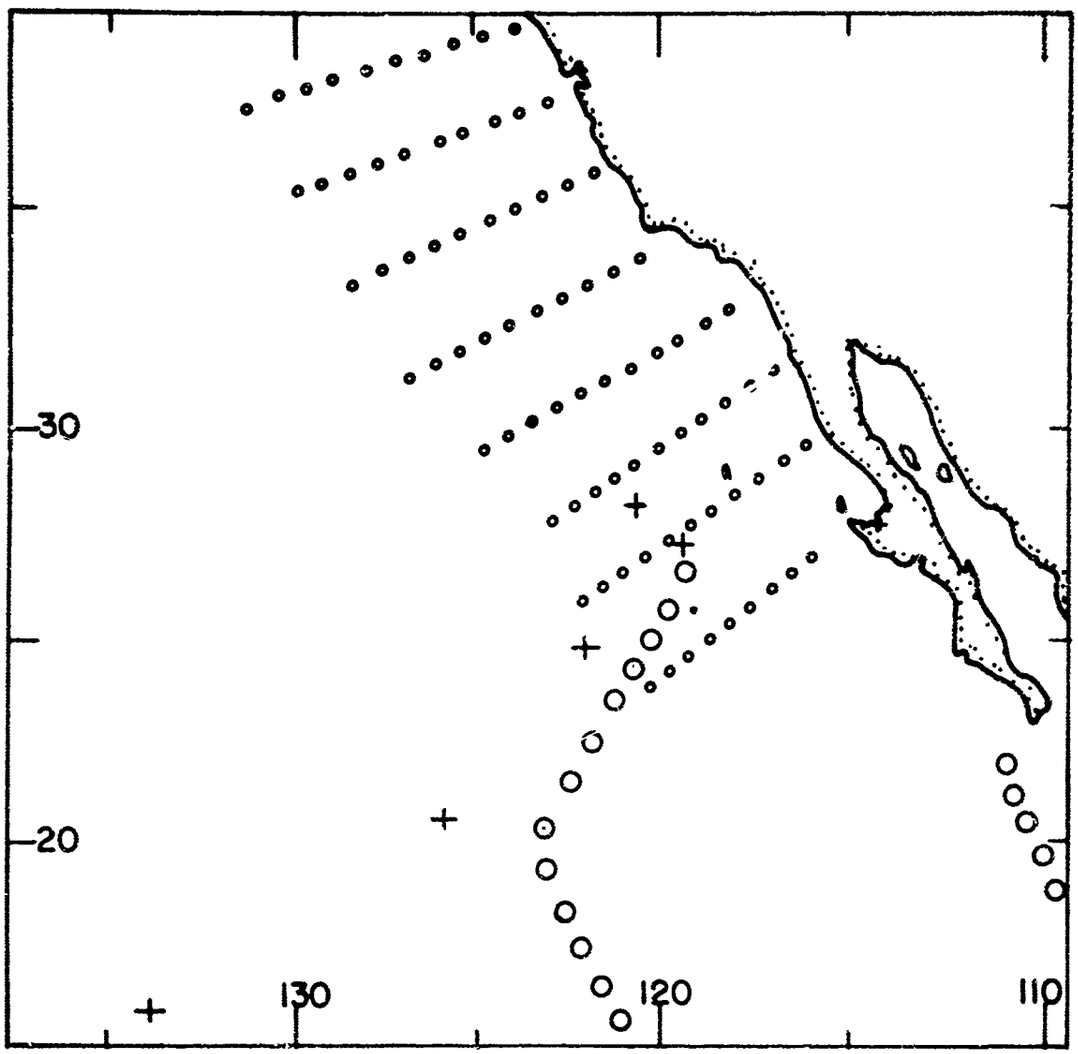

Fig. 3.1—Stations occupied for zooplankton hauls, 1949 to 1954. O, Shellback Expedition stations. +, Mid-Pacific Expedition stations. o, California Cooperative Oceanic Fisheries Investigations stations.

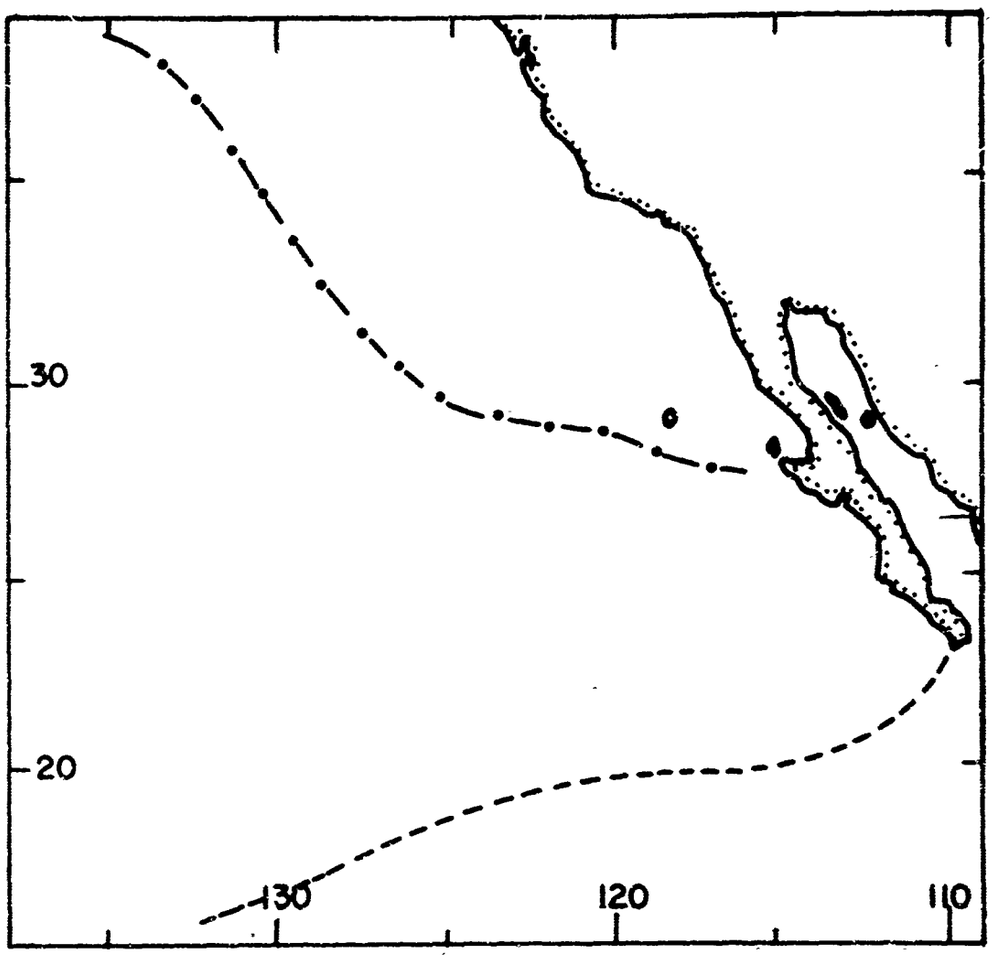

Fig. 3.2—Faunal data. •••, transition between Pacific central fauna and California current fauna. ---, break between Pacific equatorial fauna and Pacific central fauna.

18 ft in length. The intermediate-size net (0.2-mm mesh opening) was 0.5 meter in diameter and about 9 ft in length. The smallest net (0.08-mm mesh opening) had a mouth about 8 in. in diameter and was about 3 ft in length. The 1-meter nets, unless rigged for opening and closing work, had current meters suspended within them to determine the amount of water filtered. The 0.5-meter nets also were equipped with current meters.

Plankton volumes were determined by measuring the amount of water in a graduate before and after the zooplankton had been selved out. All samples were preserved in hexamine-buffered Formalin. Counts of total zooplankton and of the species composition of the Chaetognatha and Euphausiacea were made in the laboratory, usually at a magnification of 12×.

3.3 POSTTEST METHODS

The methods used immediately before and after the test were essentially the same as those mentioned above except for the following changes: A table set on shock-mounted gimbals was used for sorting and counting procedures at sea. An enclosed counting tray (plankton tray for use under way) was used successfully for the first time, making it possible to examine, at magnifications up to 36× while under way, the quality and quantity of plankton in the water. With this device the zooplankton counts could be made about as rapidly as on land and with nearly as great precision. Results obtained by this method are directly comparable to the results obtained on shore because the two methods are nearly identical.

Nylon plankton nets were used at the test and proved very satisfactory from the standpoints of strength, water-filtering capacity, and constancy of mesh size. The nets used in radioactive water came up with activity readings as high as 5000 counts/min (roughly 1 mr). Dragging the net behind the ship at 10 knots for 1 hr in uncontaminated water did not reduce the radiation significantly.

From the standpoints of time available and volume of zooplankton present, and considering the patchy distribution of the radiation, it seemed that the 1-meter net would give the best possible sample; hence it was the chief zooplankton-collecting instrument used after the test.

One opening-closing net tow was made precisely in a layer of radioactivity by attaching a 1-meter net just above the vertical telemetering probe and opening and closing the net while the probe was kept in the thin layer of activity (8 meters thick). With this technique it was possible to record the depth of tow, the temperature of the water at the depth of tow, and the radiation in the water at the depth of tow. A similar method has since been described by B. P. Boden et al., J. Marine Research Sears Foundation, 14: 205-209 (1955).

3.4 VOLUME OF ZOOPLANKTON

Five cruises were made before the test in the months of April, May, July, and September 1954 and March 1955. The volume of macroplankton in the upper 300 meters is shown in Figs. 3.3 to 3.7. In general, the standing crop decreases the farther one is from shore. There is no detectable seasonal change. In Fig. 3.8 the 50-ml (per 1000 m^3 of water filtered) contours are summarized, and the volumes taken at the test are shown. The standing crop at the test site was slightly higher than one would have expected but cannot be regarded as significantly higher owing to the small number of test-site samples. Sixty-four samples in the pretest series were to the ocean side of the 50-ml contours. These stations had standing crops with 10- to 45-ml contours. The range of the test-sample volumes was 10 to 55 ml with an average of 27 ml. The difference between the test and pretest standing-crop volumes is shown graphically in Fig. 3.9. It is perhaps significant that the two highest volumes taken at the test site had considerably higher percentages of California current species present than Pacific central species. The low-volume samples were dominated by typically Pacific central species.

In Figs. 3.10 to 3.15 the pretest standing crops of zooplankton at various depths near the test site are shown. In general, as one would expect, the zooplankton volume decreases with depth.

(Text continues on page 38.)

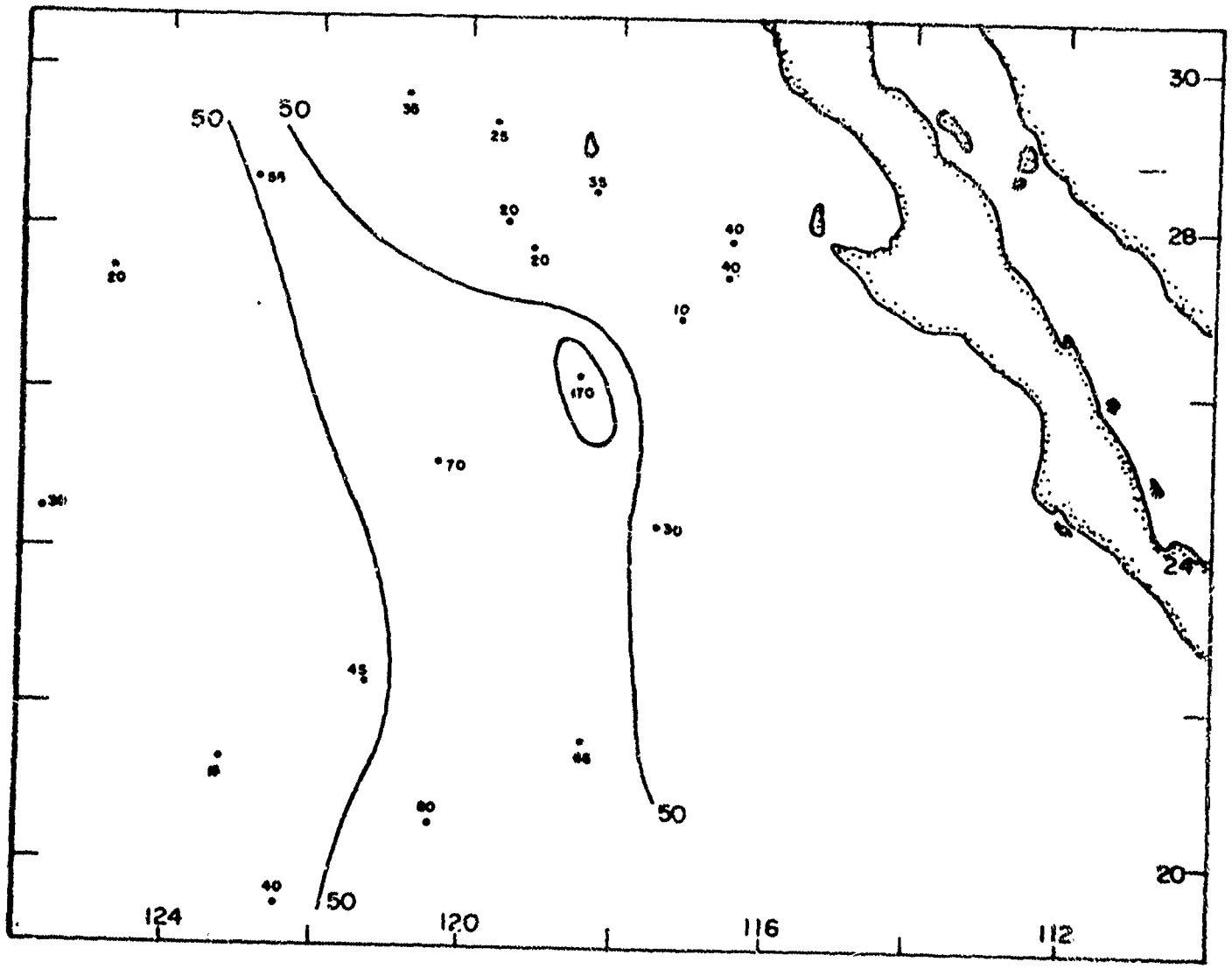

Fig. 3.3—Distribution of macroplankton volumes, Apr. 14 to May 2, 1954. The values shown are in milliliters per 1000 m^3 of water at depths from 0 to 300 meters.

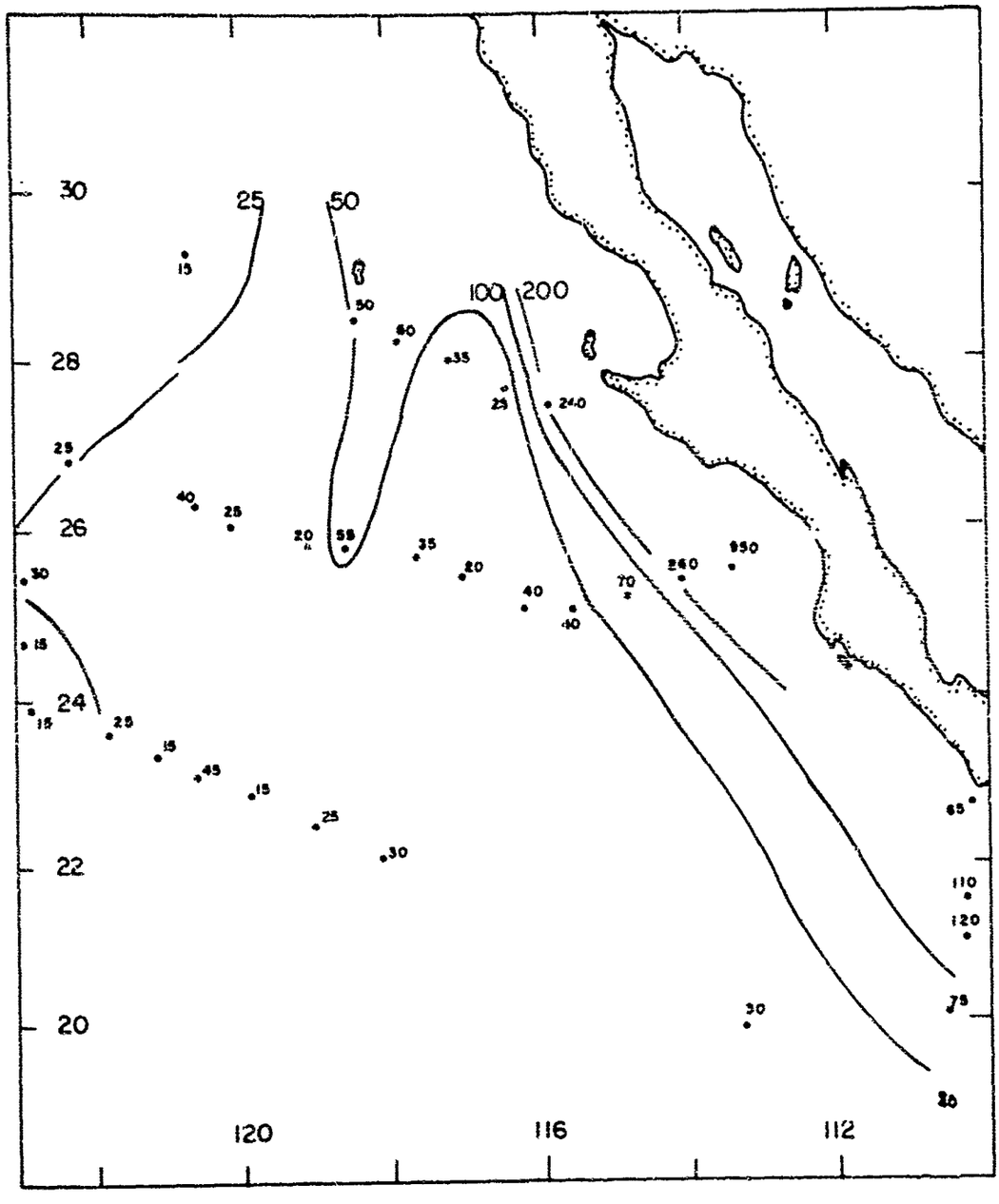

Fig. 3.4—Distribution of macroplankton volumes, May 6 to 30, 1954. The values shown are in milliliters per 1000 m^3 of water at depths from 0 to 300 meters.

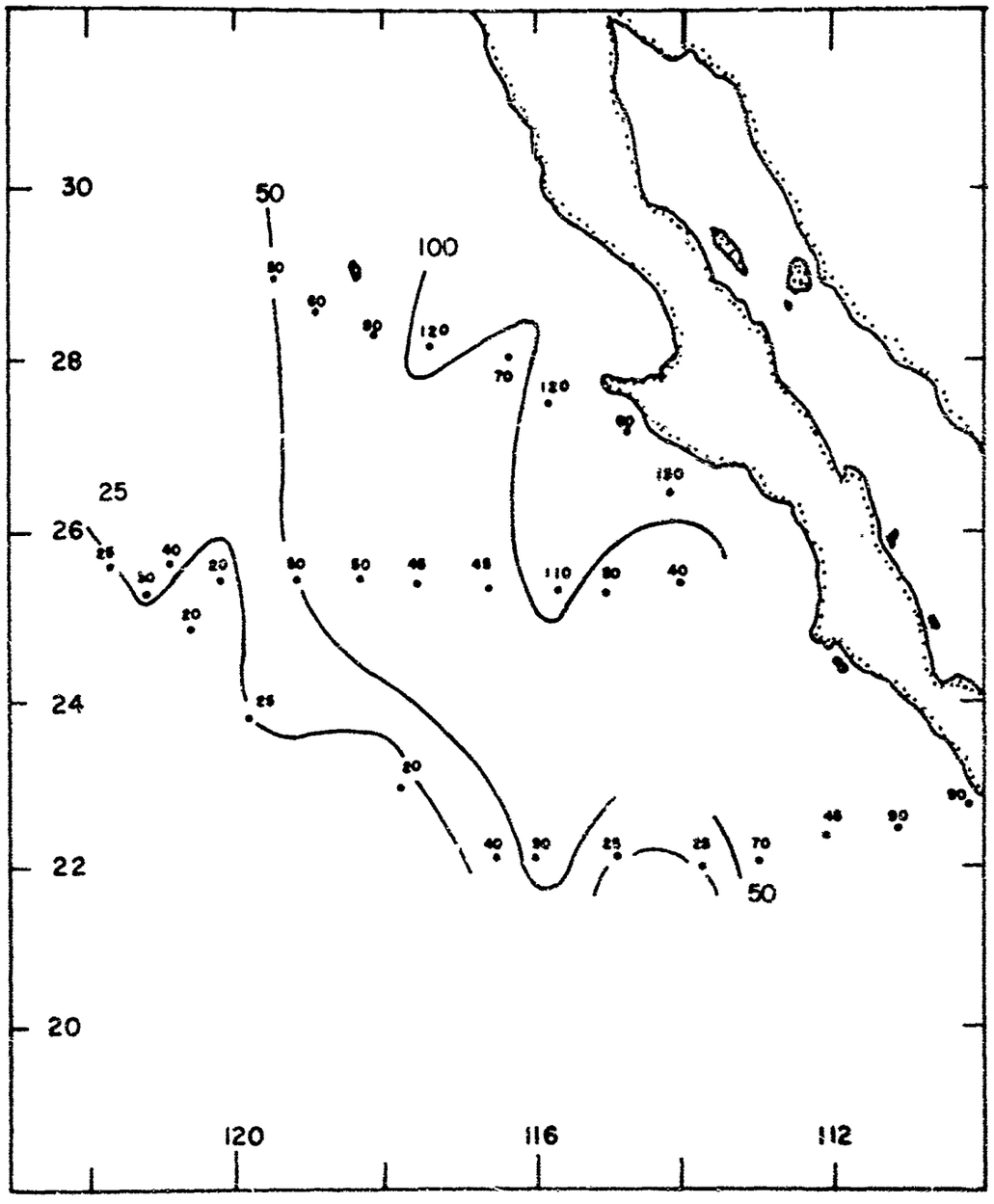

Fig. 3.5—Distribution of macroplankton volumes, July 23 to Aug. 12, 1954. The values shown are in milliliters per 1000 m^3 of water at depths from 0 to 300 meters.

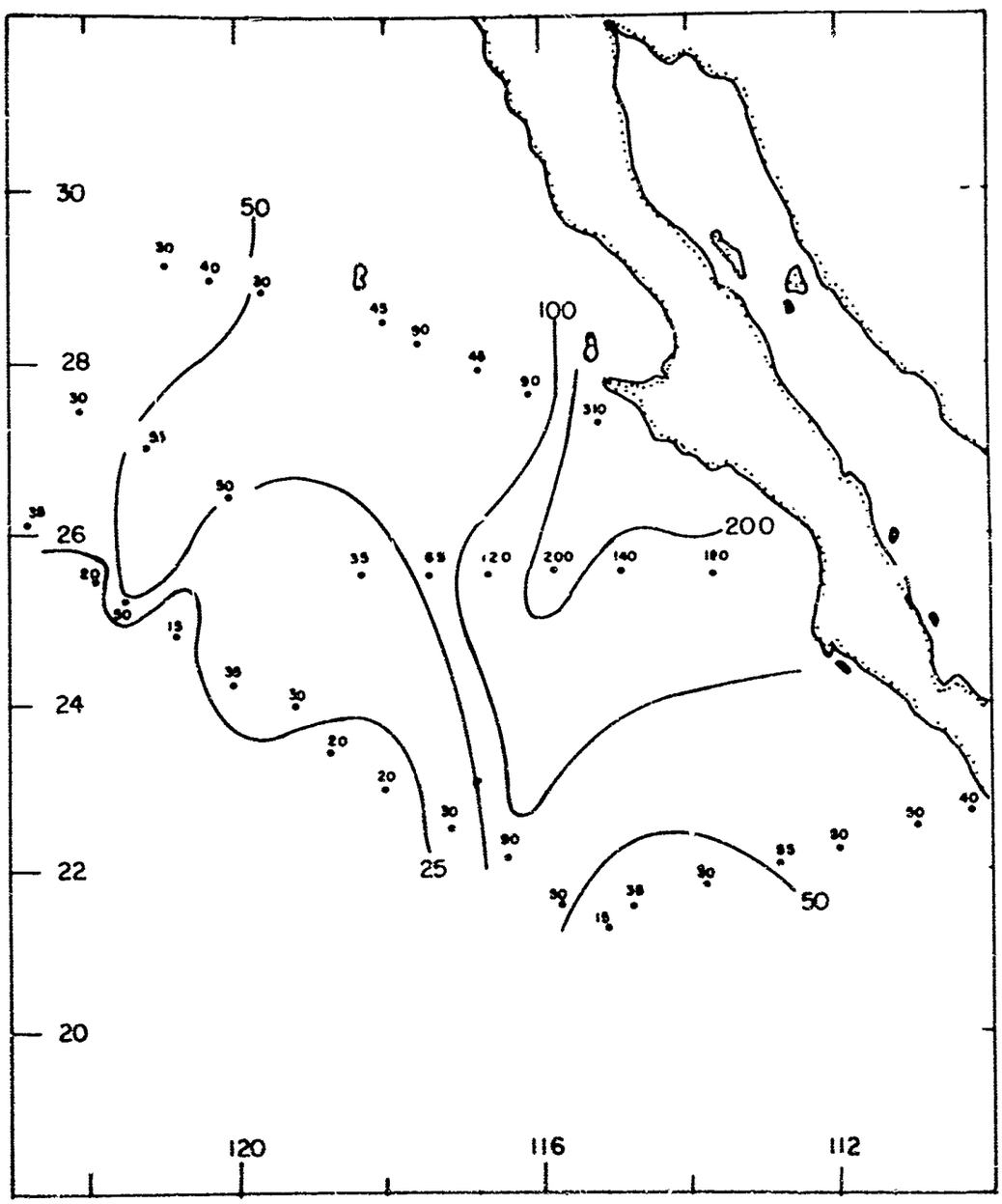

Fig. 3.6—Distribution of macroplankton volumes, Sept. 24 to Oct. 7, 1954. The values shown are in milliliters per 1000 m^3 of water at depths from 0 to 300 meters.

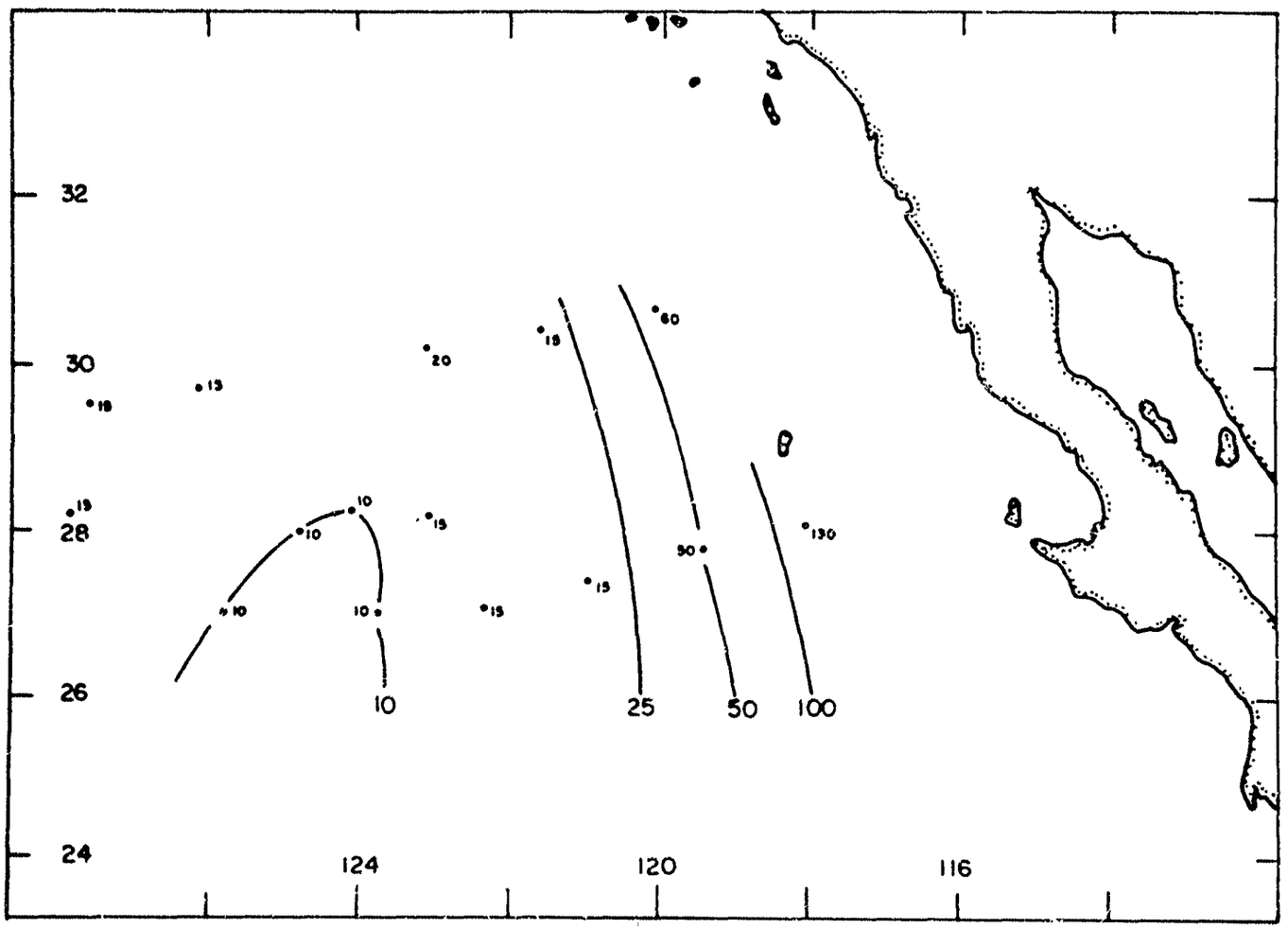

Fig. 3.7—Distribution of macroplankton volumes, Mar. 17 to Apr. 2, 1955. The values shown are in milliliters per 1000 m^3 of water at depths from 0 to 300 meters.

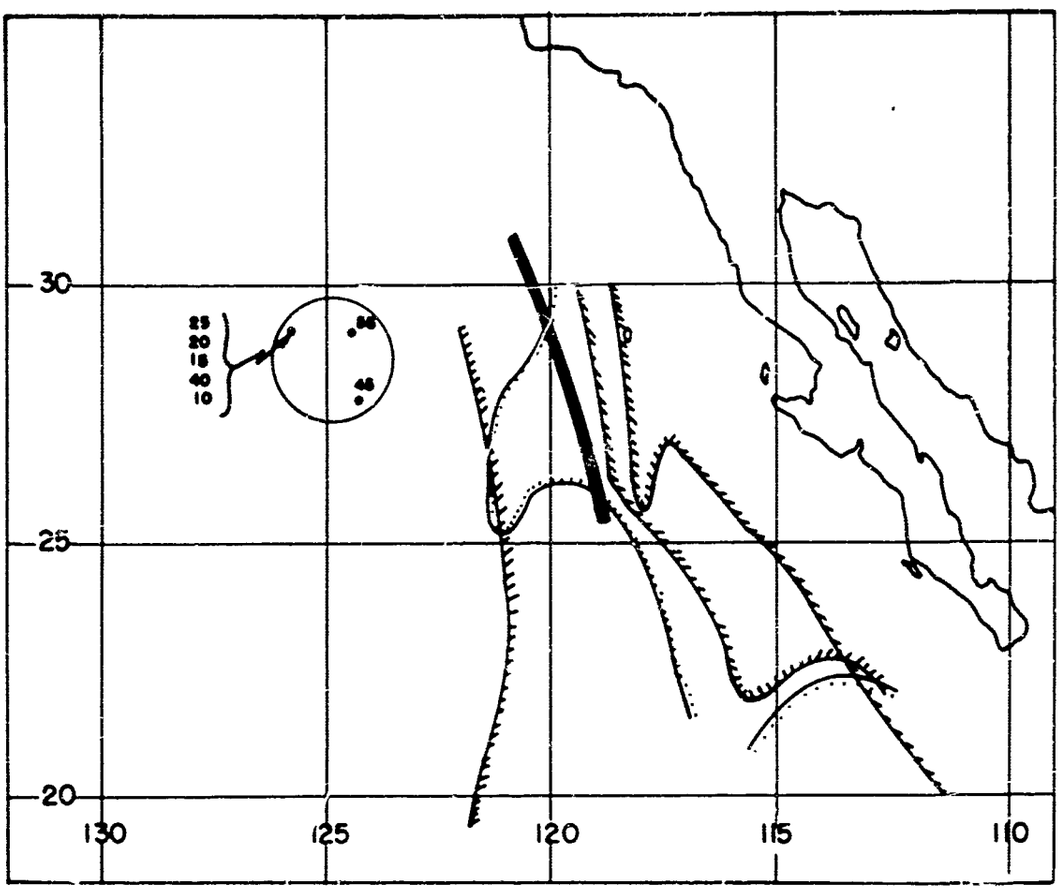

Fig. 3.8—Pretest pelagic area survey. The boundaries are for 50-ml volumes of zooplankton per 1000 m^3 of water at depths from 0 to 300 meters. West of the boundaries there are no values greater than 45 ml per 1000 m^3. (The tows of May 1955 are in the circle.)

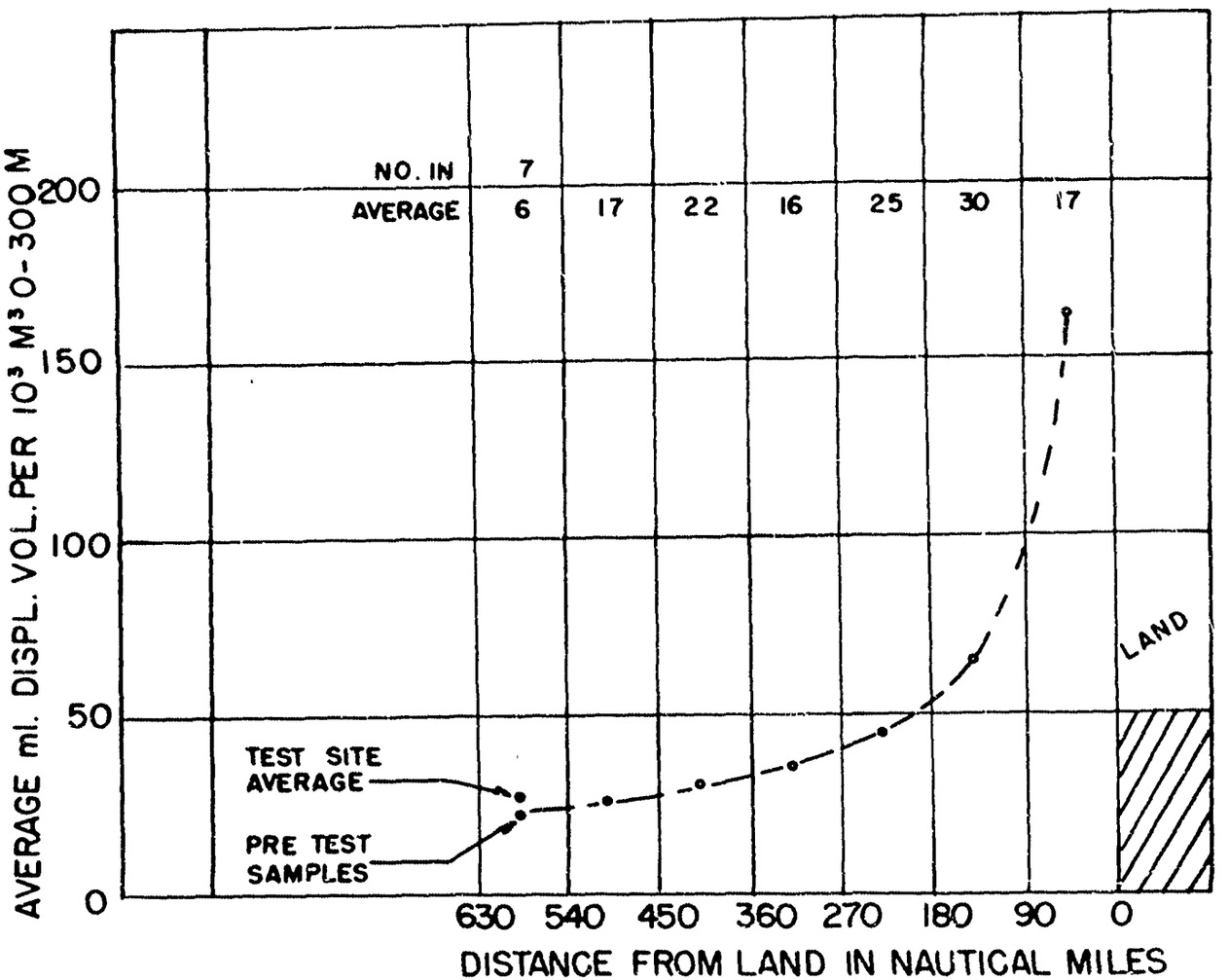

Fig. 3.9—Relation between zooplankton volumes and distance from land; also, a comparison of test and pretest zooplankton standing crops.

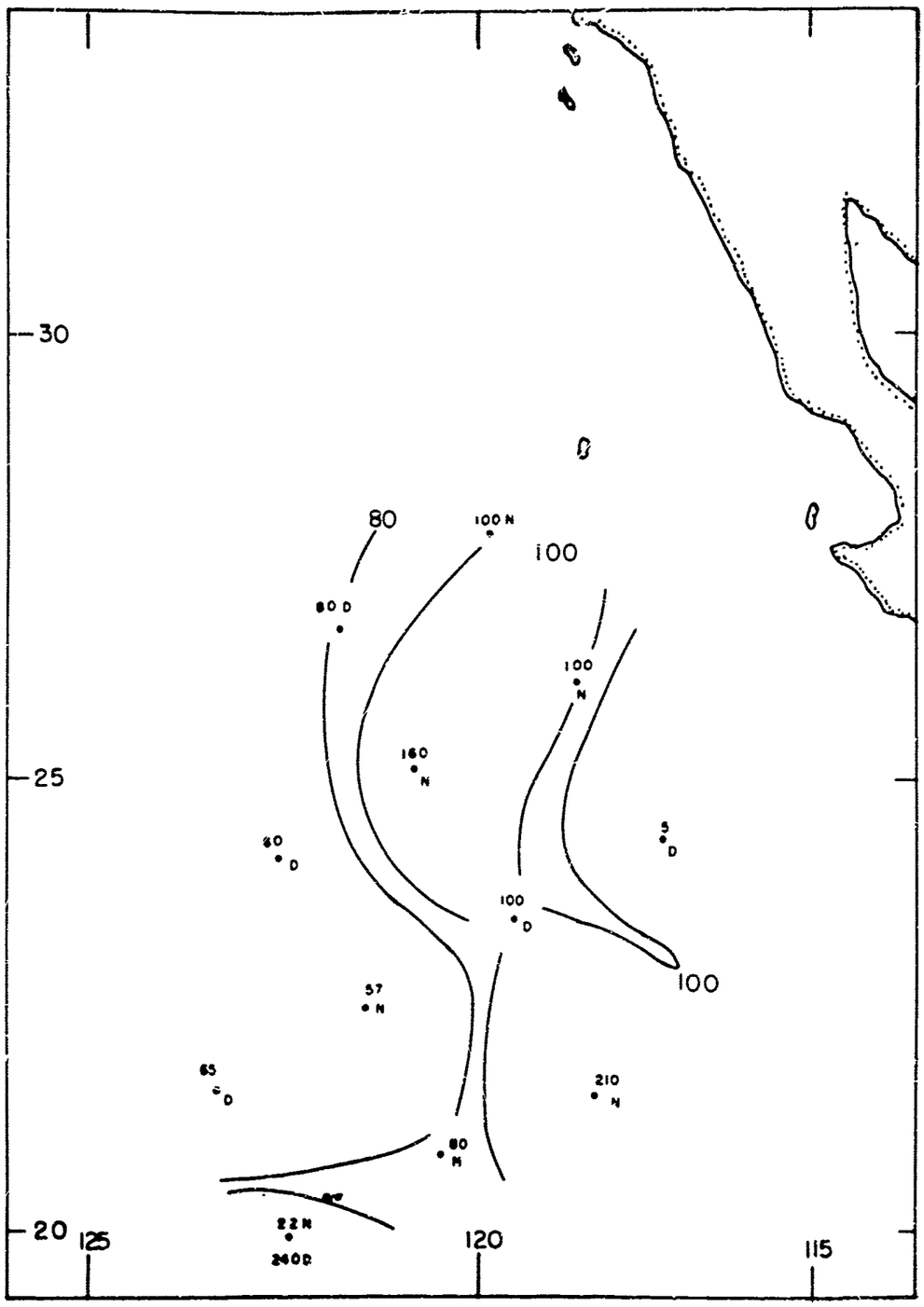

Fig. 3.10—Pretest standing crop of zooplankton near the test site at depths from 0 to 45 cm, April and May 1954. Displacement volumes are in milliliters per 1000 m^3 of water (0.5-meter net).

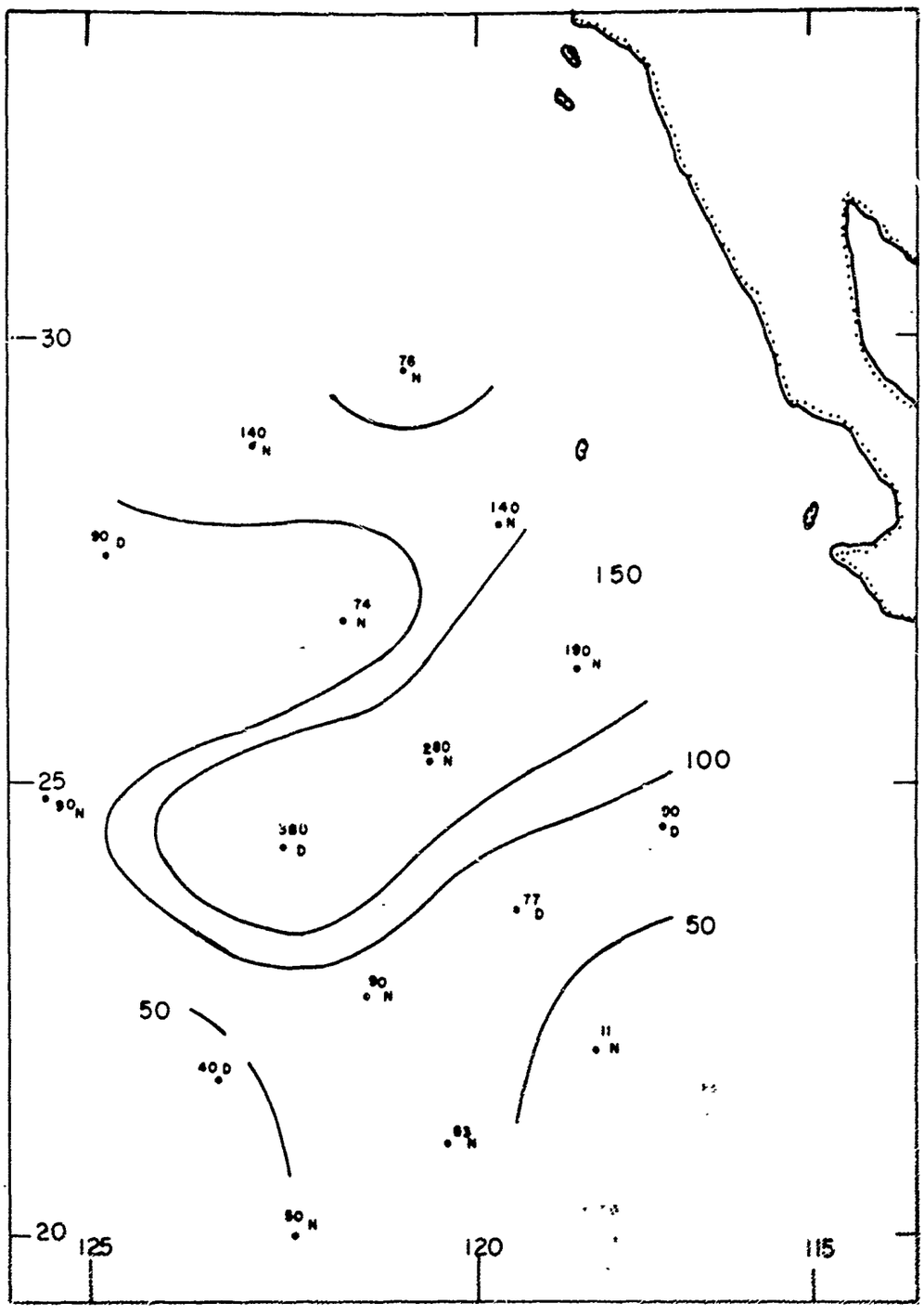

Fig. 3.11—Pretest standing crop of zooplankton near the test site at depths from 0 to 100 meters, April and May 1954. Displacement volumes are in milliliters per 1000 m^3 of water (0.5-meter net).

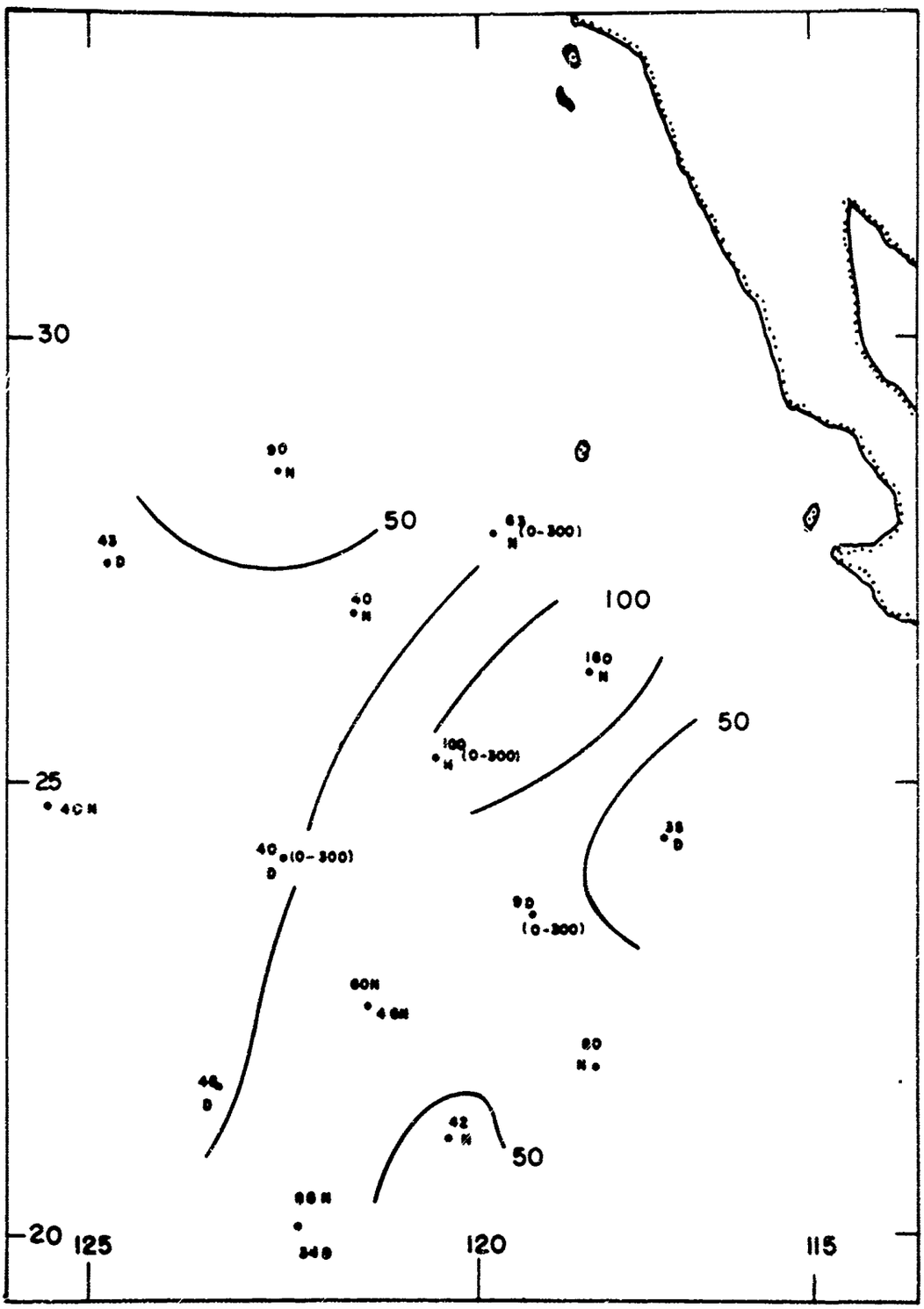

Fig. 3.12—Pretest standing crop of zooplankton near the test site at depths from 0 to 700 meters, April and May 1954. Displacement volumes are in milliliters per 1000 m^3 of water (0.5-meter net).

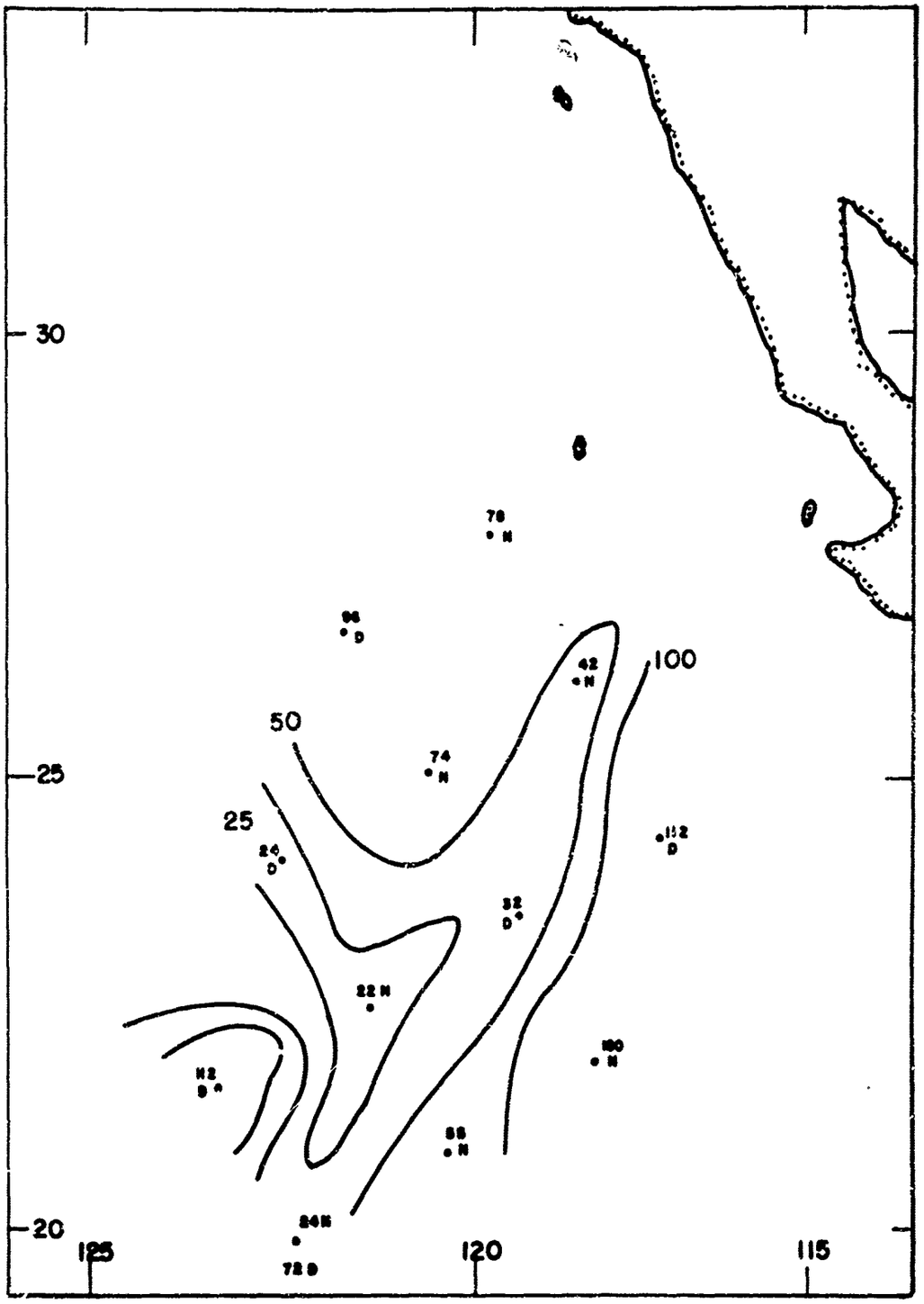

Fig. 3.13—Pretest standing crop of zooplankton near the test site at depths from 0 to 45 cm, April and May 1954. Displacement volumes are in milliliters per 1000 m^3 of water (1-meter net).

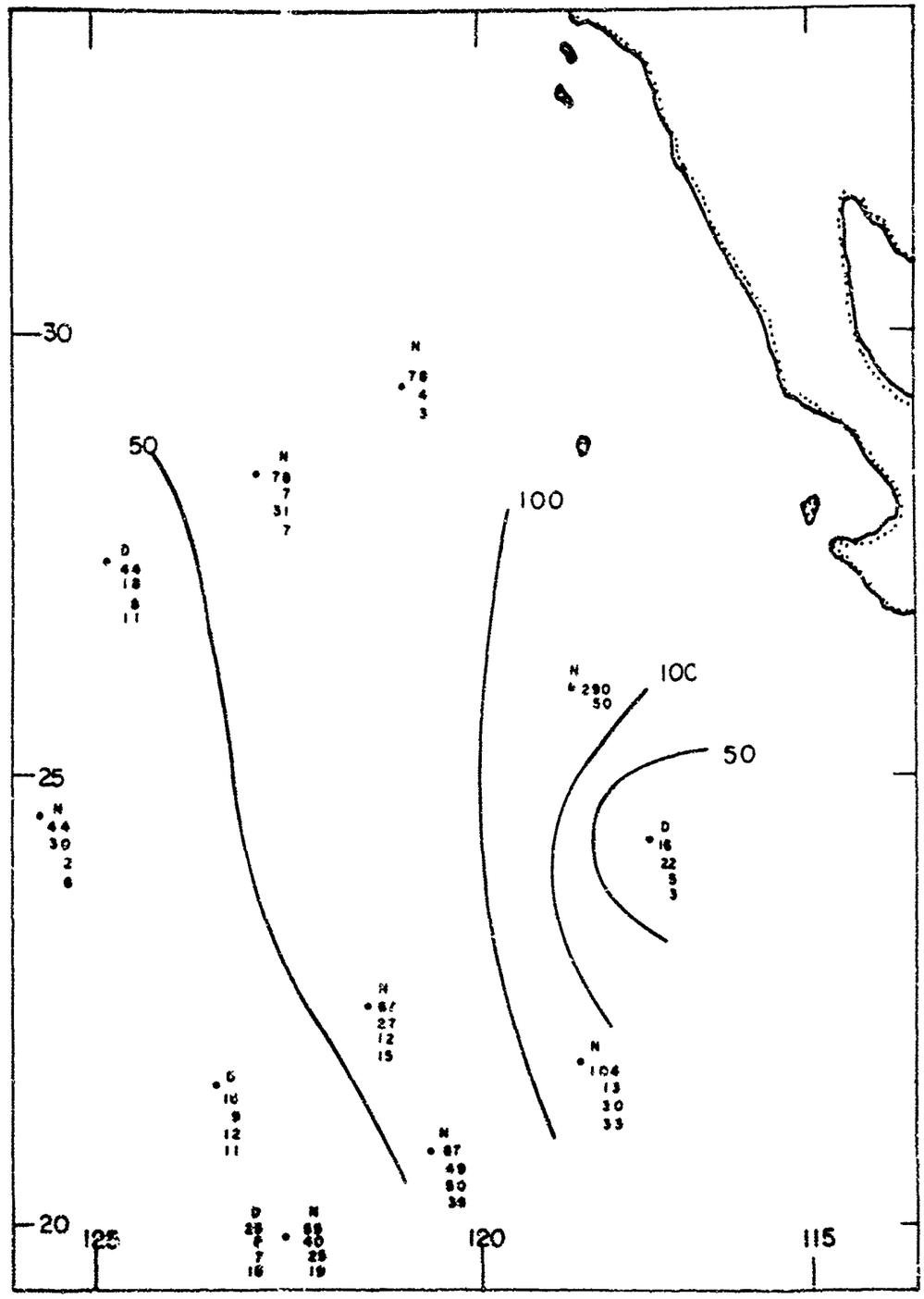

Fig. 3.14—Pretest standing crop of zooplankton near the test site at depths from 0 to 25, 25 to 50, 50 to 75, and 75 to 100 meters, April and May 1954. Displacement volumes are in milliliters per 1000 m^3 of water (1-meter net).

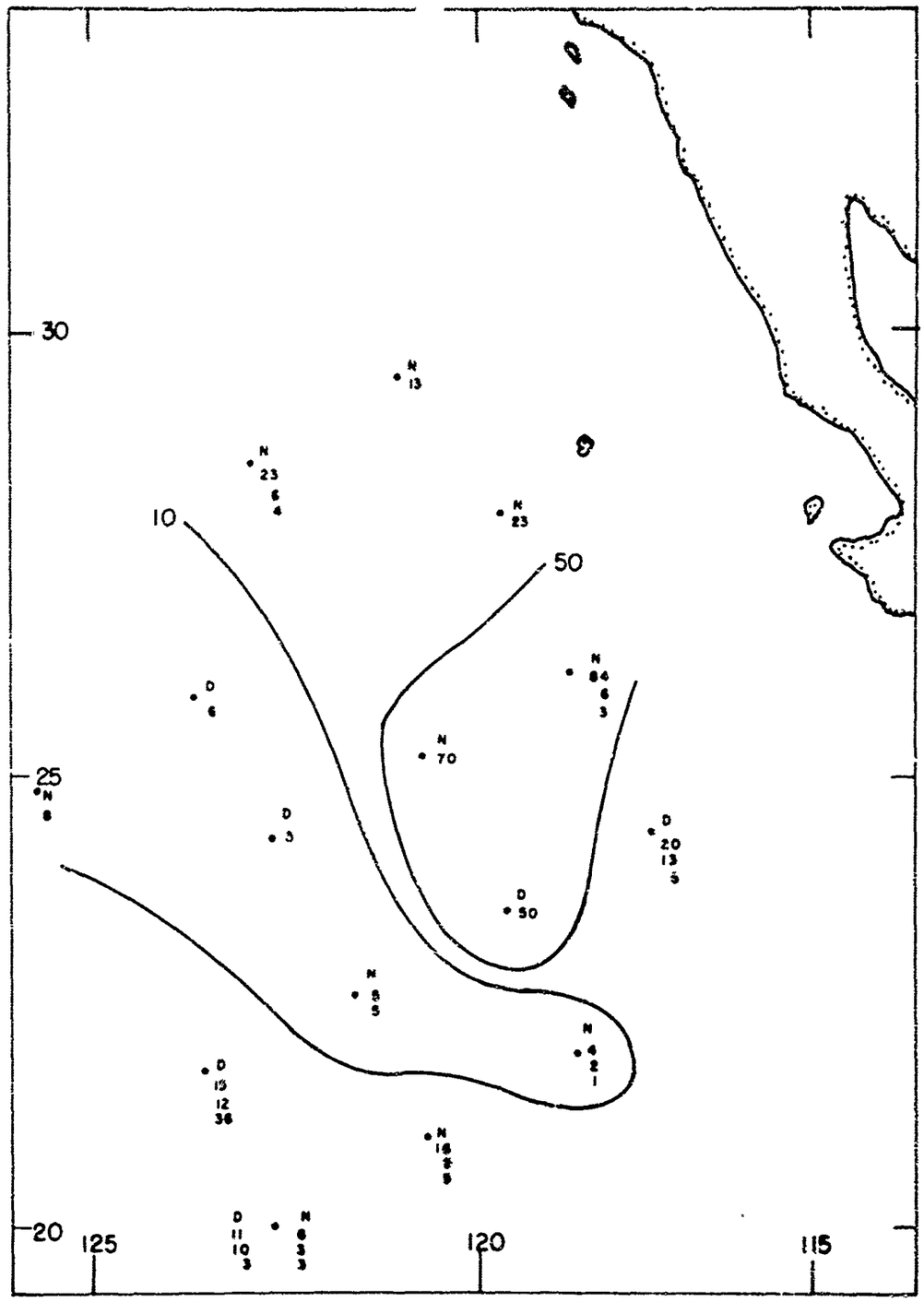

Fig. 3.15—Pretest standing crop of zooplankton near the test site at depths from 100 to 300, 300 to 500, and 500 to 700 meters, April and May 1954. Displacement volumes are in milliliters per 1000 m^3 of water (1-meter net).

2.5 ZOOPLANKTON COMPOSITION

With few exceptions copepods dominated the plankton samples, and Radiolaria, noctiluca, ostracods, chaetognaths, and euphausiids followed in abundance in that order. Salps and pteropods, the highest radiation emitters found, ranked thirteenth and seventeenth in over-all abundance. (Results of radiological examination of zooplankton organisms are given in Chap. 11.)

Six species of thecosomatous (shelled) pteropods and one species of gymnosomatous (naked) pteropods were identified and tested for activity. No single species was consistently active, but the two gymnosomatous specimens tested were both cold. The salps taken in the opening-closing net tow in the active layer were quite active and appeared to be a single species. All other salps tested (taken from other tows) were cold or only slightly active. Many other kinds of animals were found to be active, but no one group or species was consistently active.

CHAPTER 4

FISH EGGS AND LARVAE IN PELAGIC-AREA-SURVEY PLANKTON SAMPLES, 1954

By Grace L. Orton

4.1 INTRODUCTION

This chapter summarizes the data on fish eggs and larvae from the plankton samples taken on four cruises in 1954. Station plans for these cruises are shown in Figs. 4.1 to 4.3. In Tables 4.1 to 4.3 the eggs and larvae are listed by taxonomic groups (in so far as they can be identified from present knowledge), by cruise, and by approximate distance offshore.

The tables are based on a standardized list of the major taxonomic groups of teleost fishes that were found represented in the material. In addition, the list provides space for a number of other groups (notably the tunas and blennioid fishes) for which it seems desirable to emphasize absence in these collections. The tables on geographic distribution are based on a somewhat arbitrary and diagrammatic zonal pattern (Figs. 4.1 to 4.3), recording eggs and larvae occurring within areas approximately 100, 200, 300, and over 300 miles offshore.

Large numbers of eggs and moderate numbers of larvae are recorded as unidentified. The well-known difficulties of identifying marine fish eggs and larvae need not be elaborated in this report, but it should be pointed out that the bulk of the unidentified eggs is of one general type which, on further study, will probably prove referable to the stomiatoid genus Poweria, which supplies the dominant element in the larval collections.

4.2 COMMERCIAL FISHES

The PAS plankton collections contain relatively small numbers of eggs and larvae identifiable as groups of commercial interest. The occurrence of these fishes is summarized in Table 4.3, which lists numbers per sample per cruise and per 100-mile interval offshore.

One could question whether the results of this small series of cruises give an adequately representative picture of the occurrence and distribution of these fishes in the area collected. However, supporting evidence is available for some of the species from published accounts based on the very extensive Marine Life Research (MLR) collections, and this evidence gives some measure of the significance of the PAS results. Thus, the PAS data, especially on the sardine, carangid fishes, and hake, can be compared with detailed work on these fishes in recent papers by Ahlstrom,[1] Ahlstrom and Ball,[2] and Ahlstrom and Counts,[3] respectively. Additional data for comparison are given in the progress reports of CCOFI. For the most

(Text continues on page 47.)

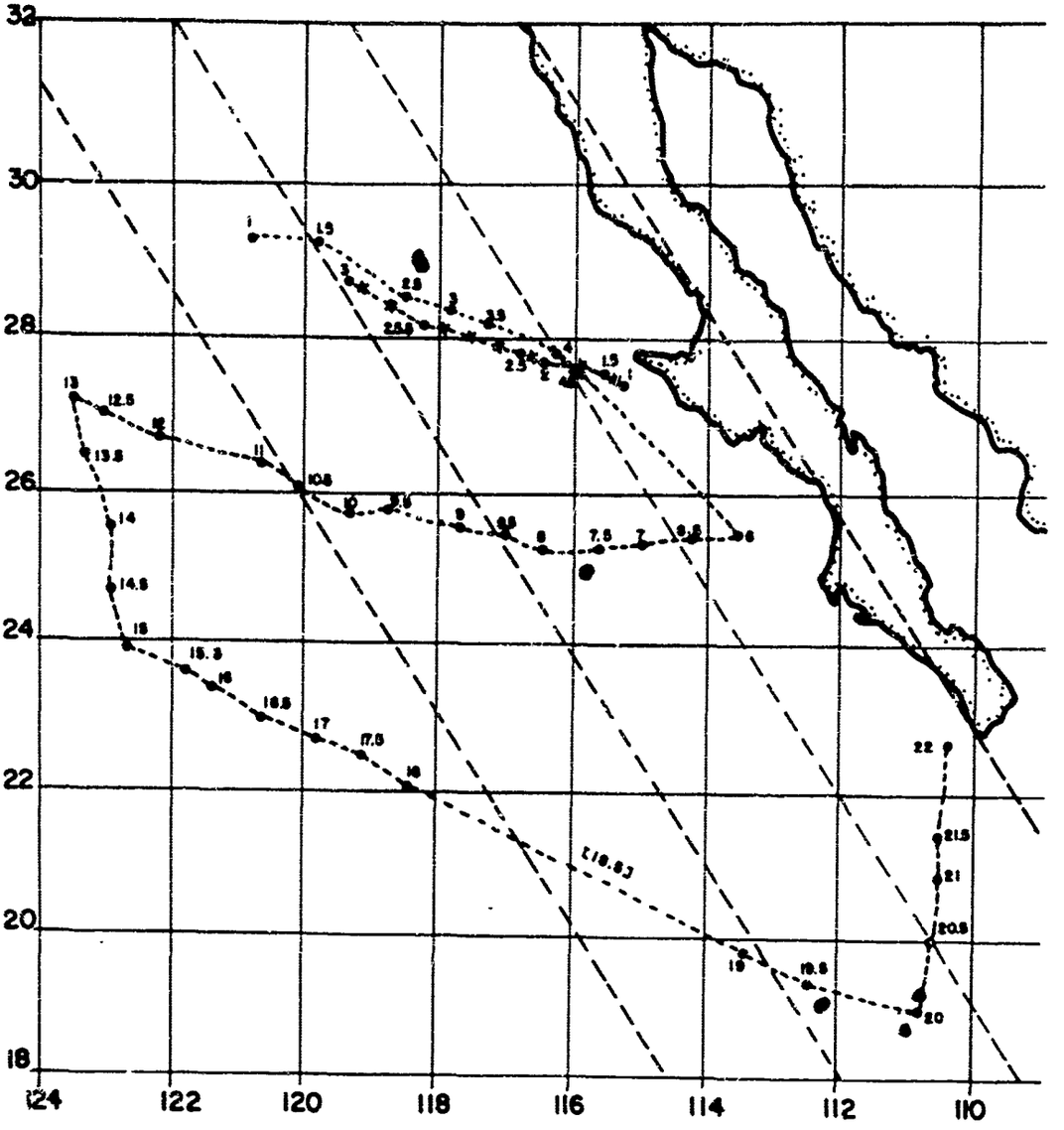

Fig. 4.1—Station plans for Paolina-T long-line cruises 1 and 2. x, cruise 1, April 1954. ●, cruise 2, May 1954.

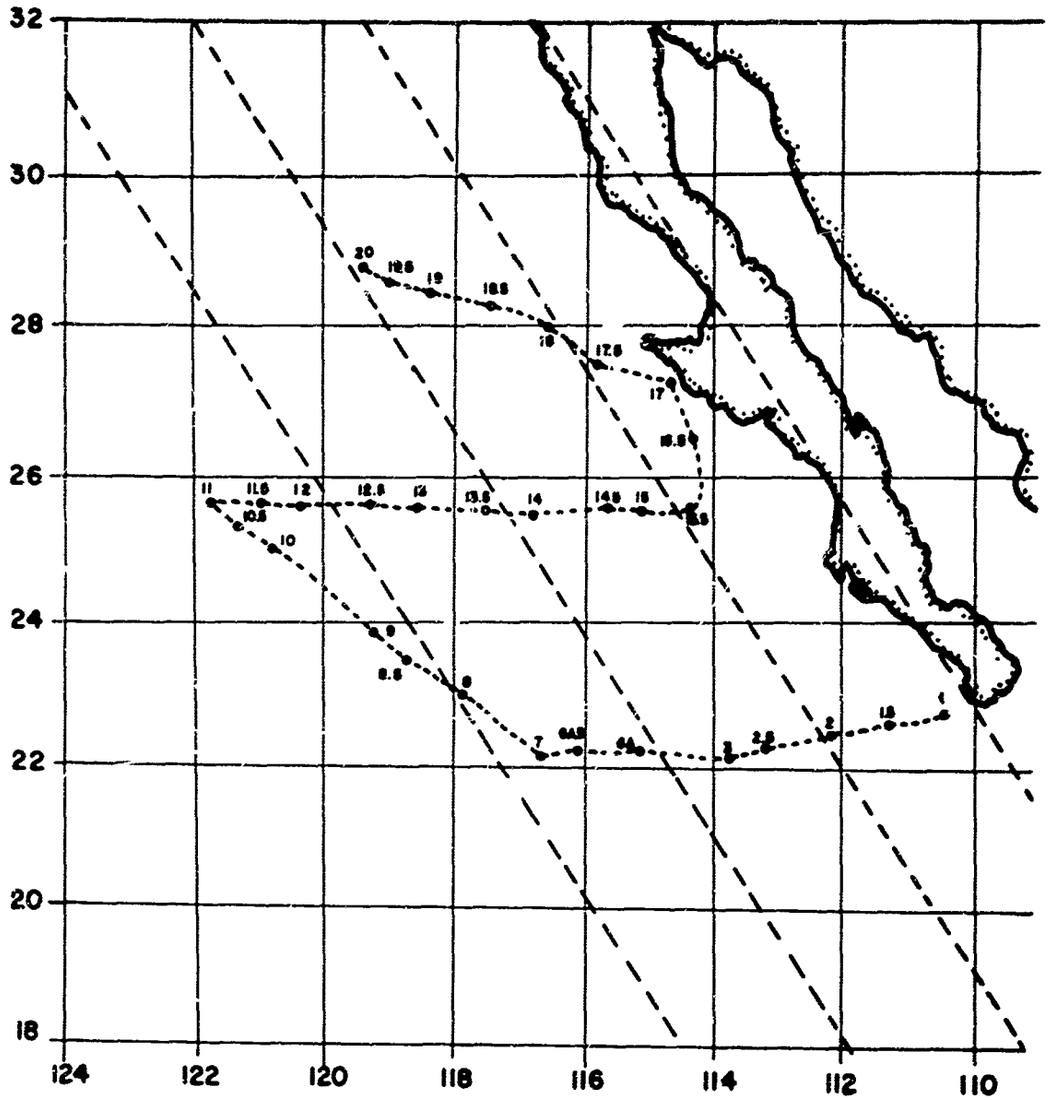

Fig. 4.2—Station plan for Paolina-T long-line cruise 3, July and August 1954.

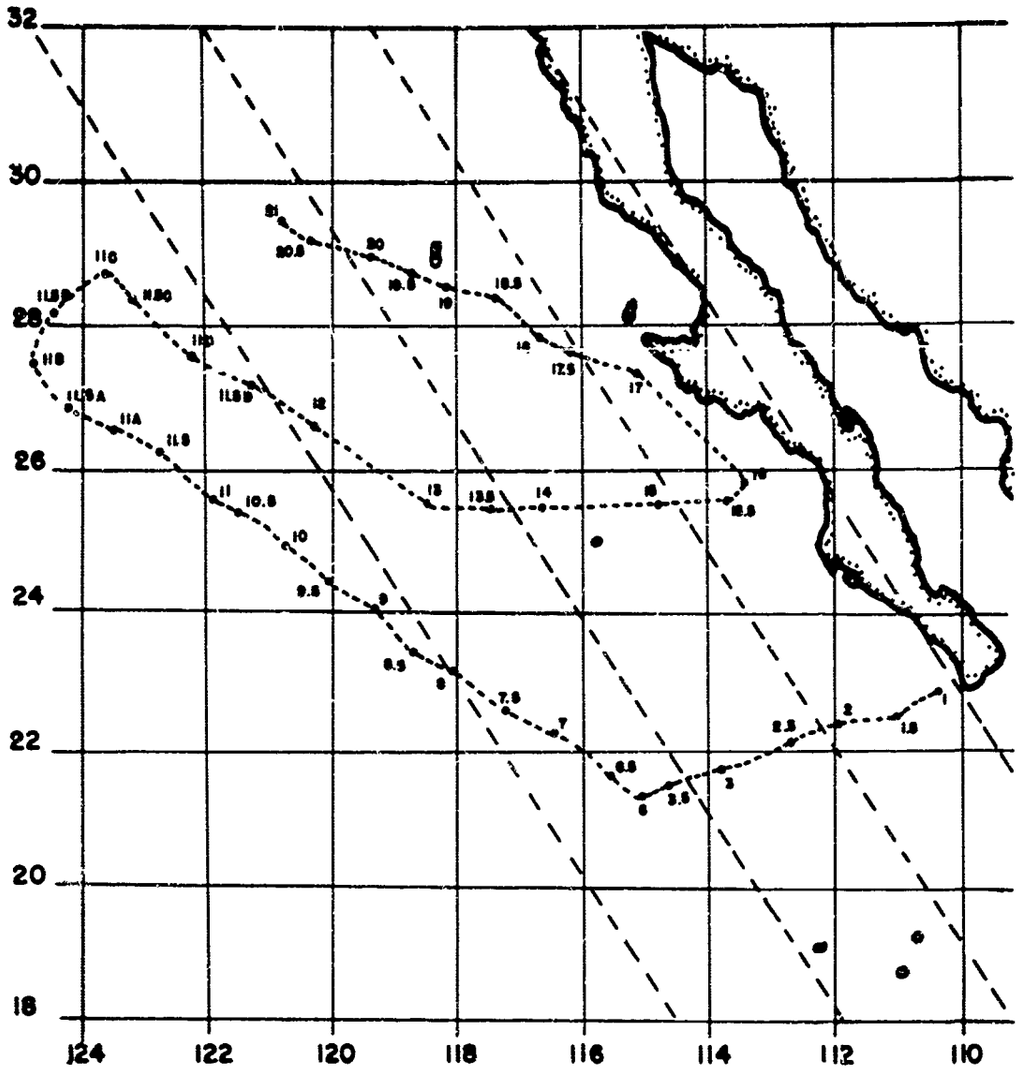

Fig. 4.3—Station plan for Paolina-T long-line cruise 4, September and October 1954.

Table 4.1 — SUMMARY OF EGGS AND LARVAE FOR ALL FOUR PAOLINA-T CRUISES
(Totals and Numbers per Sample)

	PTLL - 1 April 6 Samples				PTLL - 2 May 39 Samples			
	Eggs		Larvae		Eggs		Larvae	
	Total	Per Sample	Total	Per Sample	Total	Per Sample	Total	Per Sample
Clupeoids								
(Sardines)	52	8.66	20	3.33	7	.179	25	.641
(Anchovies)			549	91.5			85	2.17
Alepocephalids					2	.051		
Argentinids	422	70.33	49	8.16	376	9.64	124	3.17
Eels	1	.166					3	.076
Stomiatoids			13	2.16	2	.051	1377	35.307
Myctophiids			56	9.33			884	22.64
Inioscus Fishes			5	.83			19	.487
Synentognaths								
(Saury)	1	.166			3	.076	1	.025
(Flyingfish)					3	.076		
Cod-like Fishes								
(Hake)	45	7.5	132	22.0			16	.415
(Macrourids)								
(Misc.)							3	.076
Allotriognaths					4	.102		
Flatfishes	1	.166	307	51.16			16	.415
Berycoids								
(Melamphaeids)			2	.33			7	.179
Typical Percoids								
(Serranids)								
Muziloids								
(Barracuda)								
Carangiforms								
(Carangids)	11	1.83	24	4.0	190	4.87	65	1.66
(Dolphins)					5	.125		
Scombriforms								
(Mackerel)					2	.051	1	.025
(Tuna)								
(Gempylids, etc.)	2	.33					1	.025
Bramids							1	.025
Tetragonurus								
Scorpaeniforms								
(Scorpaenids)			167	27.83			26	.666
(Cottids)			1	.166				
(Triglids)								
Chiasmodontids					8	.205	8	.205
Callionymids								
Blennioids								
Gobies								
Ceratioids								
Unidentified	283		20		7508		139	

Table 4.1 — (Continued)

	PTLL - 3 July - August 31 Samples				PTLL - 4 September - October 42 Samples			
	Eggs		Larvae		Eggs		Larvae	
	Total	Per Sample	Total	Per Sample	Total	Per Sample	Total	Per Sample
Clupeoids							1	.023
(Sardine)	6	.193	24	.774	1	.023		
(Anchovies)	4	.129	54	1.74			2	.047
Alepocephalids	1	.032						
Argentinids	312	10.06	44	1.41	72	1.714	63	1.50
Eels			2	.064				
Stomiatoids	7	.222	1887	60.87	11	.261	3793	90.30
Myctophids			762	24.58			1092	26.00
Inicmous Fishes			23	.741			28	.666
Synentognaths								
(Saury)								
(Flyingfish)			1	.032	5	.119		
Cod-like Fishes								
(Hake)								
(Macrourids)							1	.023
(Misc.)			2	.064			1	.023
Allotriognaths	7	.222			7	.166		
Flatfishes			6	.193			42	1.0
Berycoids								
(Melamphaeids)			17	.548			21	.5
Typical Percoids			39	1.25			13	.309
(Serranids)					98(?)	2.33		
Mugiloids								
(Barracuda)			1	.032				
Carangiforms								
(Carangids)					17	.404	2	.047
(Dolphins)	15	.483	3	.096				
Scombriforms								
(Mackerel)	2	.064	1	.032				
(Tuna)					143	3.40	4	.095
(Gempylids, etc.)	326	10.51	1	.032	14	.333	6	.142
Bramids	13	.419	2	.064			1	.023
Tetragonurus								
Scorpaeniforms			2	.064			1	.023
(Scorpaenids)			9	.290				
(Cottids)			5	.161				
(Triglids)			1(?)	.032	1	.023	12	.285
Chiasmodontids	3	.096	11	.354			12(?)	.285
Callionymids								
Blennioids							2	.047
Gobiss							5	.119
Ceratioids								
Unidentified	7460		99		2971		172	

Table 4.2—SUMMARY OF EGGS AND LARVAE TABULATED BY ZONES OFFSHORE FOR ALL FOUR PAOLINA-T CRUISES COMBINED
(Totals and Numbers per Sample)

	100 Miles, 21 Samples				200 Miles, 36 Samples			
	Eggs		Larvae		Eggs		Larvae	
	Total	Per Sample	Total	Per Sample	Total	Per Sample	Total	Per Sample
Clupeoids								
(Sardine)	52	2.47	55	2.619	11	.305	14	.388
(Anchovies)	4	.190	495	23.47			5	.139
Alepocephalids	2	.095			1	.0277		
Argentinids	343	16.33	48	2.285	718	19.94	196	5.44
Eels			2	.095	1	.0277	1	.0277
Stomiatoids	2	.095	511	24.33	3	.0833	1412	39.22
Myctophids			530	25.23			779	21.63
Inicmous Fishes			4	.190			12	.333
Synentognaths								
(Saury)					3	.0833	1	.0277
(Flyingfish)	7	.333			1	.0277		
Cod-like Fishes								
(Hake)	40	1.904	117	5.57	5	.139	31	.861
(Macrourids)							1	.0277
(Misc.)							2	.055
Allotriognaths	3	.142			9	.250		
Flatfishes	1	.047	334	15.904			35	.972
Berycoids								
(Melamphaeids			5	.238			17	.472
Typical Percoids			31	1.476			11	.305
(Serranids)	98(?)4.66							
Muziloids								
(Barracuda)			1	.047				
Carangiforms								
(Carangids)					110	3.05	53	1.47
(Dolphins)	28	1.33	1	.047	2	.055		
Scombriforms								
(Mackerel)	2	.095	1	.047				
(Tuna)								
(Gempylids, etc.)	464	22.09	3	.142	4	.111	1	.0277
Bramids					12	.333	1	.0277
Tetragonurus							1	.0277
Scorpaeniforms							2	.055
(Scorpaenids)			194	9.238			9	.250
(Cottids)			6	.285				
(Triglids)			1(?)	.047				
Chiasmodontids	1	.047	2	.095	6	.166	7	.194
Callionymids								
Blennioids								
Gobies			2	.095				
Ceratioids								

Table 4.2 — (Continued)

	300 Miles, 24 Samples				Over 300 Miles, 36 Samples			
	Eggs		Larvae		Eggs		Larvae	
	Total	Per Sample	Total	Per Sample	Total	Per Sample	Total	Per Sample
Clupeoids								
(Sardine)	1	.0416			1	.0277		
(Anchovies)								
Alepocephalids								
Argentinids	93	3.87	34	1.41	55	1.52	12	.333
Eels							2	.055
Stomiatoids	5	.208	2060	85.83	10	.277	3028	84.11
Myctophids			578	24.08			806	22.38
Iniomous Fishes			15	.625			44	1.22
Synentognaths								
(Saury)								
(Flyingfish)			1	.0416				
Cod-like Fishes								
(Hake)								
(Macrourids)								
(Misc.)							3	.0833
Allotriognaths	2	.0833			4	.111		
Flatfishes			1	.0416				
Berycoids								
(Melamphaeids)			13	.541			11	.305
Typical Percoids			4	.166			6	.166
(Serranids)								
Muziloids								
(Barracuda)								
Carangiforms								
(Carangids)	37	1.54	16	.666	54	1.50	25	.694
(Dolphins)	7	.291	4	.166				
Scombriforms								
(Mackerel)	2	.0833	1	.0416				
(Tuna)								
(Gempylids, etc.)	3(?)	.125	1	.0416			1	.0277
Bramids	5	.208	3	.125	10	.277	5	.139
Tetragomurus								
Scorpaeniforms								
(Scorpaenids)								
(Cottids)								
(Triglids)								
Chiasmodontids	4	.166	18	.750	1	.0277	3	.0833
Callionymids			3(?)	.125			9(?)	.250
Blennioids								
Gobies								
Ceratioids			1(?)	.0416			5	.139

Table 4.3—SUMMARY OF EGGS AND LARVAE OF COMMERCIAL GROUPS AND OF SOME OF THE DOMINANT NONCOMMERCIAL GROUPS

	Numbers Per Sample Per Cruise				Numbers Per Sample Per Zone Offshore			
	6 Samples	39 Samples	31 Samples	42 Samples	21 Samples	36 Samples	24 Samples	36 Samples
	PTLL-1 April	PTLL-2 May	PTLL-3 July - August	PTLL-4 Sept.- October	100 Miles	200 Miles	300 Miles	Over 300 Miles
Sardine eggs	8.66	.179	.193	.023	2.47	.305	.0416	.0277
Sardine larvae	3.33	.641	.774	-	2.619	.388	-	-
Anchovy eggs	-	-	.129	-	.190	-	-	-
Anchovy larvae	91.5	2.17	1.74	.047	23.47	.39	-	-
Saury eggs	.166	.076	-	-	-	.0833	-	-
Saury larvae	-	.025	-	-	-	.0277	-	-
Hake eggs	7.5	-	-	-	1.904	.139	-	-
Hake larvae	22.0	.415	-	-	5.57	.861	-	-
Flatfish larvae	51.16	.415	.193	1.0	15.904	.972	.0416	-
Barracuda larvae	-	-	.032	-	.047	-	-	-
Carangid eggs	1.83	4.87	-	-	-	3.05	1.54	1.50
Carangid larvae	4.0	1.66	-	-	-	1.47	.666	.694
Dolphin eggs	-	.125	.483	.404	1.33	.055	.291	-
Dolphin larvae	-	-	.096	.047	.047	-	.166	-
Pacific Mackerel								
eggs	-	.051	.064	-	.095	-	.0833	-
larvae	-	.025	.032	-	.047	-	.0416	-
Scorpaenid larvae	27.83	.666	.290	.023	9.238	.250	-	-
Non-Commercial Groups								
Argentinid eggs	70.33	9.64	10.06	1.714	16.33	19.94	3.87	1.52
Argentinid larvae	8.16	3.17	1.41	1.50	2.285	5.44	1.41	.333
Stomiatoid larvae	2.16	35.307	60.87	90.30	24.33	39.22	85.83	84.11
Myctophid larvae	9.33	22.64	24.58	26.00	25.23	21.63	24.08	22.38

part the PAS findings are in rather close agreement with published MLR results, but the scorpaenid larvae show a considerable departure. This indicates the need for caution in interpreting the rather limited data obtained by PAS.

4.2.1 Sardines

Sardine eggs were taken on all four PAS cruises, and larvae were taken on all but cruise 4. The eggs were most abundant on cruise 1 (April); total numbers of larvae taken on cruises 1 to 3 were relatively constant (Table 4.1). The actual total numbers taken per cruise appear to be more significant for comparison than the numbers per sample per cruise, since the definitely identifiable sardine material was all taken north of 25° north latitude regardless of cruise track and number of samples. Both eggs and larvae were most abundant within the first 100 miles offshore and fell off sharply to the seaward (Table 4.2). One egg was recorded from the 300-mile zone and one from over 300 miles.

The PAS data on sardine eggs and larvae closely approximate the MLR results in both seasonal and geographic occurrence. Ahlstrom (reference 1, page 106) reported: "Spawning off southern California and adjacent Baja California may occur at considerable distances from shore. Sardine eggs have been collected about 250 miles offshore, and larvae at even greater distances; but large concentrations of eggs are seldom taken farther from shore than 150 miles." In summarizing data on the spawning season, he reported (reference 1, page 139): "Sardine eggs have been collected during every month of the year off central Baja California, although the period of major abundance has been limited to the months of February through May."

4.2.2 Anchovies

Some anchovy larvae were taken on all four PAS cruises, but eggs were obtained only on cruise 3. The larvae were very abundant on cruise 1 (April), and the numbers fell off sharply and progressively on the later cruises. Nearly all the larvae (495) were taken within the 100-mile zone, very few (five) in the 200-mile zone, and none farther offshore (see Table 4.2).

Information concerning the distribution of anchovy larvae taken on the MLR cruises in 1951 and 1952 was mapped and briefly discussed in Sardine Progress Reports for 1952 (page 40) and 1953 (pages 34 and 35). Although the larvae were more abundant at inshore stations in both years, some occurred as far as 300 miles offshore in 1951 and as far out as 200 miles in 1952. In 1951, spawning off central Baja California was most abundant from February through May, but some spawning occurred throughout the year. It is evident that anchovies can occur substantially farther offshore than the PAS cruises found them.

4.2.3 Hake

Hake eggs and larvae were taken only on the first two PAS cruises, predominantly on cruise 1 (April). None were taken farther offshore than 200 miles and none farther south than 27° north latitude.

These results accord closely with those of the more extensive MLR studies. Ahlstrom and Counts[3] found hake larvae abundant only from February to April; in both 1951 and 1952, about 98 per cent of all hake larvae were taken in this period. In both years about 68 per cent were taken in the area from Point Conception to San Quintin, Baja California. Off southern California they were taken as far as 350 miles offshore, but in both years the area of concentration narrowed to the southward to a zone much closer inshore (see especially reference 3, Figs. 12 and 13).

4.2.4 Jack Mackerel

Carangid eggs and larvae were taken only on the first two PAS cruises (April and May). They were most abundant in the 200-mile zone, with lesser numbers at 300 miles and over 300 miles. Furthermore, none were taken south of 24° north latitude. It is possible that all the carangid eggs and larvae taken during the PAS are jack mackerel, but, since early stages of this fish have not yet been clearly differentiated from related carangids that might occur in the area (e.g., species of Decapterus), the more conservative course of simply calling them carangids is followed in this report.

The occurrence of carangid eggs and larvae in the PAS plankton accords well with the MLR results on jack-mackerel larvae. Ahlstrom and Ball (reference 2, page 225 et seq.) found that, in the MLR samples for 1950 and 1951, jack-mackerel larvae were concentrated in a broad area well offshore from central California southward to central Baja California, with small numbers taken as far south as the level of Magdalena Bay in 1951 (the 1950 cruises did not extend that far south). The larvae were most abundant in a broad area about 80 to 240 miles offshore. Larvae continued to appear in samples as far seaward as the MLR cruises operate, although there was a steady decrease in abundance outward beyond 240 miles. The larvae were most abundant during the period of March through July; about 98 to 99.5 per cent of the larvae were taken during this five-month period.

4.2.5 Scombroid Fishes

No eggs or larvae identifiable as tuna were found in the PAS plankton. However, some of the other scombroid fishes are represented. Very small numbers of eggs and larvae of Pacific mackerel were taken on cruises 2 and 3 (May to August), occurring then in the 100-mile and 300-mile zones (see Tables 4.1 and 4.3). The numbers are too small to be very meaningful directly, but it is of interest to compare them with the data on Pacific mackerel larvae taken by MLR in 1952 (Sardine Progress Report, 1953, Fig. 26, pages 38 and 39). That report also indicates the relatively small numbers of larvae taken and their spotty distribution as far seaward as 300 miles. Off central Baja California on the 1952 MLR cruises, the larvae were found to be most abundant in April and May, but some spawning occurred during most of the year. Eggs and larvae of at least one species of gempylid are represented in the PAS collections (see especially Tables 4.1 to 4.3). Large numbers of gempylid eggs were obtained on cruises 3 and 4 from inshore stations near and southward from Point Eugenio, in the general region that closely corresponds to an important area of upwelling mapped in the Sardine Progress Report for 1953 (Fig. 4, page 10).

4.2.6 Miscellaneous Commercial Fishes

Several additional teleost groups that include species of commercial or sport fishing interest are represented in the PAS plankton collections. Flatfish larvae were taken on all four cruises but with a heavy predominance on cruise 1. Nearly all (334) of the total were taken within the 100-mile zone, only 35 in the 200-mile zone, and only one in the 300-mile zone. Their heavy concentration inshore is a reasonable expectation for flatfishes in general. The data suggest some seasonal concentration, but the flatfishes comprise a large group of species with poorly known life histories, and the several species represented among the PAS larvae may well differ significantly in spawning season. Hence it seems unwise to speculate on seasonal occurrence of this composite group from limited data.

The samples contain a single barracuda larva, taken on cruise 3, station 17. This locality is close inshore south of Point Eugenio. There is too little information available on the spawning of barracudas in Mexican waters to warrant interpretation of the possible significance of this single specimen (a recently hatched larva).

Small numbers of dolphin eggs and larvae were taken, chiefly on cruises 3 and 4. The eggs were most abundant in the 100-mile zone, but a few were taken at 200 and 300 miles. Larvae occurred in the 100- and 300-mile zones. There has apparently been no work published on the early life history of the dolphins in the eastern Pacific. The seasonal and zonal distribution of the PAS material suggests that spawning of these fishes may be associated with relatively warm water and may extend to moderate distances offshore.

At least two kinds of scorpaenid larvae are present in the collections. They probably represent species of Scorpaena and Sebastodes. Most of these larvae were taken on cruise 1 (April), and most were from the 100-mile zone. These limited data suggest sharp seasonal and offshore limitations, but the much more extensive MLR data apparently do not bear this out. Thus the Sardine Progress Report for 1952 (Fig. 51, page 43) maps the scorpaenid larvae collected in 1951 and records that they were widely distributed in every month and that they were taken persistently as far as 300 miles offshore. More extensive year-by-year comparisons are needed before the apparent difference between the MLR data for 1951 and the PAS data for 1954 can be evaluated.

4.3 NONCOMMERCIAL FISHES

The many groups of noncommercial fishes represented in the PAS plankton are indicated in the tables. The three groups that dominate the samples, i.e., the argentinids, stomiatoids, and myctophids, are included in Table 4.3 for comparison with the fishes that are of direct commercial interest. A stomiatoid genus, Poweria, is the most conspicuous form throughout

the larval collections. These larvae show a steady increase in numbers throughout the summer, and a heavy concentration far offshore, from 300 miles outward.

The nearly uniform distribution of myctophid larvae, both in season and in distance offshore, is noteworthy.

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2. E. H. Ahlstrom and O. P. Ball, Description of Eggs and Larvae of Jack Mackerel (Trachurus symmetricus) and Distribution and Abundance of Larvae in 1950 and 1951, U. S. Fish and Wildlife Service Fishery Bull. No. 97, 1954, pp. 209-245, Figs. 1 to 28.
3. E. H. Ahlstrom and R. C. Counts, Eggs and Larvae of the Pacific Hake, Merluccius productus, U. S. Fish and Wildlife Service Fishery Bull. No. 99, 1955, pp. 295-329, Figs. 1 to 25.

CHAPTER 5

MIDWATER TRAWL SURVEYS

By Robert L. Wisner

Trawling was done on two cruises, cruise 5406-F, June 15 to 30, 1954, and during Operation Wigwam, May 1955. All trawling was done aboard the R/V Spencer F. Baird of the Scripps Institution of Oceanography. The track of cruise 5406-F and the location of each trawl for both cruise 5406-F and Operation Wigwam are shown in Fig. 5.1. All trawling during Operation Wigwam was done in the vicinity of the detonation site and the subsequent contaminated area.

The primary purpose of cruise 5406-F was to ascertain the type of fauna present and the general abundance in a broad area between the probable detonation site and the shore waters endangered by contamination by radioactive material resulting from the detonation. A secondary purpose was to study the availability of marine organisms that could form a food potential for commercially important fishes, primarily the tunas. The total area sampled exceeded the pelagic area as outlined in Fig. 5.1 in order to estimate the total distribution of organisms.

The Isaacs-Kidd 10-ft midwater trawl was used exclusively as the sampling instrument. It was fished at depths ranging from 0 to 1944 fathoms. The trawl was modified from the original by adapting the end to accommodate a 1-meter silk plankton net for use as a cod end. This permits retaining a portion of the very small organisms gathered by the net. A liner of ½-in. stretch mesh was placed inside the 2½-in. stretch mesh of the main trawl net. Thus it was possible to sample a wide size range of organisms.

During cruise 5406-F a total of 15 trawls was made. Table 5.1 lists the duration and depth of each trawl. Since the trawl net is always open, it is assumed to be sampling from the surface to its greatest depth. Therefore the depths are listed from the surface (0) to the maximum depth attained, given as fathoms.

During Operation Wigwam a total of five trawls was made to provide specimens to determine the radiological background count prior to detonation of the bomb and to determine the amount of specimen contamination following detonation. No track is given since maneuvering was intermittent and random. The trawls are listed, as for cruise 5406-F, in Table 5.2.

Cruise 5406-F has been completely studied in regard to the fishes taken. A total of 1763 specimens was taken, comprising 38 genera and 20 families. Table 5.3 lists all specimens by family, genera, and species.

All fishes taken during Operation Wigwam have not been studied in detail. Those studied for uptake of radioactive substances will be listed in Chap. 12 of this report. The remaining fishes indicate no significant faunal differences from the specimens listed in Table 5.3. No specimen list is given for fishes taken during Operation Wigwam.

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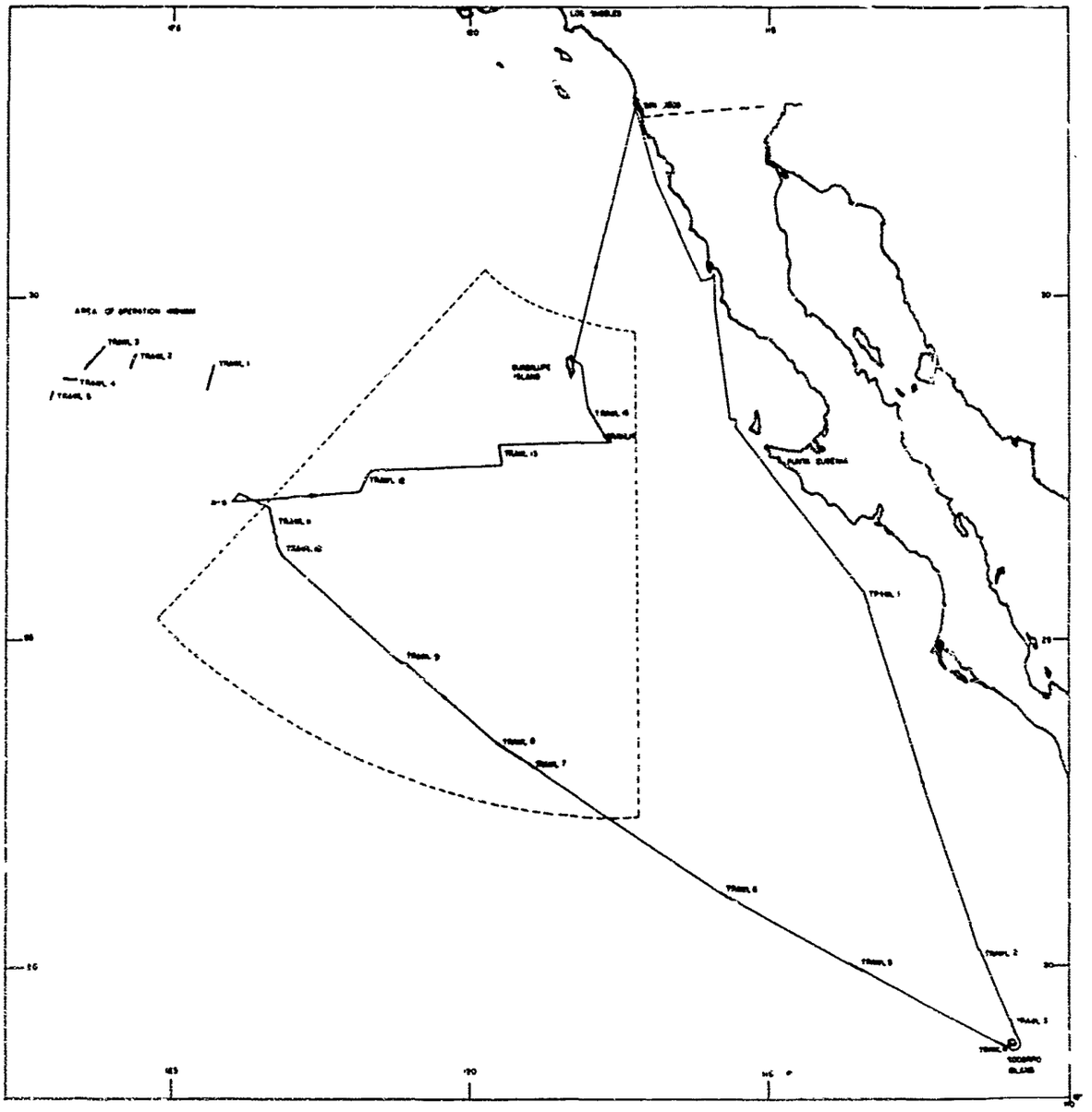


Fig. 5.1—Midwater trawl work prior to and during Operation Wigwam.

Table 5.1 — TRAWLS MADE DURING CRUISE 5406-F

The trawl numbers correspond with those shown on the cruise track in Figure 5.1

Trawl	Duration in Hours-Minutes	Depth in Fathoms
1	3 - 55	0 -93
2	4 - 30	0 -75
3	6 - 40	0 -875
4	4 - 32	0 -83
5	4 - 25	0 -100
6	4 - 00	0 -93
7	2 - 57	0 -96
8	10 - 40	0 -1163
9	3 - 58	0 -103
10	4 - 29	0 -100
11	10 - 02	0 -1578
12	4 - 40	0 -72
13	5 - 01	0 -93
14	4 - 17	0 -93
15	9 - 45	0 -1498

Table 5.2 — TRAWLS MADE DURING OPERATION WIGWAM

The numbers correspond with those shown in Figure 5.1

Trawl	Duration in Hours-Minutes	Depth in Fathoms
1	11 - 10	0 -1944
2	4 - 40	0 -412
3	8 - 50	0 -412
4	9 - 30	0 -348
5	4 - 10	0 -150

Table 5.3—NUMBERS PER SPECIES OF FISH TAKEN DURING CRUISE 5406-F IN PELAGIC AREA WATERS, JUNE 15 TO 30, 1954

Family / Genus and Species	Trawls Taken														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Alepocephalidae															
Leptochilichthys agassizi															1
Argentinodae															
Bathylagus nigrigenys			3	6					2				11	3	
Bathylagus wesethi									2				11	3	3
Bathylagus sp. nov.															1
Microstoma microstoma									1						
Sternoptychidae															
Danophos oculatus											3				
Diplophos taenea		1	5												
Sternoptyx obscura								4							1
Argyropelecus beathi								2	3						
Argyropelecus lychnus		3	1				1		1		3			2	1
Argyropelecus affinis								2			4				7
Powerie lucetia		22	33	57		3	4		6						3
Cyclothone signata								11	9	4	32	16	11		3
Cyclothone acclinidens			100	20				66	26	3	156	25			70
Stomiatidae															
Stomias atriventer		5	4	11			1						3	1	1
Idiacanthidae															
Idiacanthus antrostomus				1				2					1		
Myctophidae															
Benthosema panamense								1							
Diaphus pacificus		3	43	5											
Diaphus andersoni									4						
Diogenichthys laternatus		30	13	9				1						2	1
Diogenichthys atlanticus											1				
Ceratoscopelus townsendi							15	6	8				1		3
Hygophum reinhardti		2	6	1			1								1
Lampanyctus festivus														3	1
Lampanyctus idostigma		14	5				14	3	8						2
Lampanyctus omostigma	1			4											
Lampanyctus mexicanus		4	3				13	1		1	1		31	65	68
Lampanyctus pyrsobolus			2	2			3		1				1		
Lampanyctus regalis							1		6		2		2		
Lampanyctus niger									1						
Lampadena bathyphila											1				
Lampadena sp. nov.							1		1						
Electrona artica								1			1				
Scopelengyidae															
Scopelengys tristis															2
Melanostomiatidae															
Bathophilus indicus									1						
Bathophilus filifer				10											
Eustomias sp.									1						
Leptostomias sp.									1						
Evermannellidae															
Evermannella indica							3		1						
Paralepidae															
Lestidium ringens													2	3	
Lestidium sp.									1						
Profundisudis coruscanus									2				1		
Scopelarchidae															
Scopelarchis analis		7							1	2					

Table 5.3 — (Continued)

Family / Genus and Species	Trawls Taken														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Nemichthyidae															
Nemichthys scolopaceus		1								1	1	1			
Avocettina bowersi			1								1				
Cyema atratum											1		1		
Merlucciidae															
Merluccius sp.														31	1
Anoplogastridae															
Anoplogaster cornuta															1
Cadidae															
Microlepidium grandiceps		7		26											
Melamphaidae															
Melamphaes bispinosus		239	21	61					55	4			8		2
Melamphaes mycterinus			1								1				
Melamphaes sp. nov.		1	2						1				4		1
Bragmaceratidae															
Bragmaceros macclellandi		2	3	1											
Bothidae															
Citharichthys xanthostigma														3	1
Pleuronectidae															
Microstomus pacificus														2	
Brotulidae															
Unidentifiable			1												
Total species per trawl	1	15	18	14		1	11	12	24	6	14	3	15	11	22
Total specimens per trawl	1	341	247	214		3	57	100	153	15	208	42	89	118	175

Total specimens identified	1763
Total genera	38
Total families	20

Of the fishes taken during both cruises, none were found to be other than indigenous to the normal deep-sea environment. No migratory or commercial fishes were encountered. The numbers of fishes per trawl, both absolute and in species, were notably lower than for trawls of comparable depth and duration at localities nearer coastal waters.

This substantiates the original findings of a general paucity of life in the area. This paucity was exemplified in the scarcity of surface life. No marine mammals were noted, except in coastal waters, and the only bird life consisted of the wide-ranging family of storm petrels. Even these were infrequently seen.

CHAPTER 6

RESULTS OF LONG-LINE FISHING BY M/V PAOLINA-T

By B. M. Shimada

6.1 PRETEST SURVEY

One of the prime considerations respecting the biological aspects of Operation Wigwam was the possible effect of the test upon the pelagic fish resources of the test area, particularly those of commercial importance. Although little information was available on the character and extent of this fish fauna, it was known that some species of tunas at least were to be found in this general area during the summer and fall months and that at this time the area supported a fairly extensive fishery. Therefore, in order to obtain first-hand information on the biota of the area of interest, to assist in selecting the time and site of the final test, and, at the same time, anticipating possible repercussions from commercial fishery interests in the posttest period for which data needed to be gathered, a series of fishing cruises was commenced in May 1954, fully a year before the event, by the M/V Paolina-T of the Scripps Institution of Oceanography. The primary mission of these cruises, of which there were four between May 1954 and April 1955, was to determine, by means of long-line fishing and other sampling, the seasonal and geographical distribution and abundance of edible fishes in the general oceanic region from Guadalupe Island south to the Revillagigedo Islands and seaward from the Mexican mainland to approximately 128° west longitude.

A pattern of stations bracketing the Wigwam area was established for each cruise, with special emphasis in the final cruises upon the northern sector southwest of Guadalupe Island. At each station, fishing was carried on with long lines, which are long lengths of cotton lines buoyed at the surface and from which baited hooks are hung to fish at different levels in the sea. A total of 76 sets was made during the year's time, equivalent to 20,718 hooks fished, which resulted in the capture of 666 fishes of various kinds. The catches consisted predominantly of sharks but also included a few tunas, spearfishes, and other miscellaneous species (Table 6.1). Of these, the tunas and four swordfish that were taken in the course of the survey were the only fishes of commercial value. Figure 6.1 shows the track taken by each cruise and the stations where tunas were caught on the long lines. The catch rates per 100 hooks fished per day by cruise were as follows:

	Tuna	Spearfish	Sharks	Misc.	All species
Cruise 1 (May 3–June 4, 1954)	0.12	0.04	2.45	0.32	2.93
Cruise 2 (July 13–Aug. 14, 1954)	0.12	0.77	1.30	0.49	2.68
Cruise 3 (Sept. 14–Oct. 19, 1954)	0.11	0.31	1.09	0.34	1.85
Cruise 4 (Mar. 11–Apr. 4, 1955)	0	0	6.30	0.13	6.43

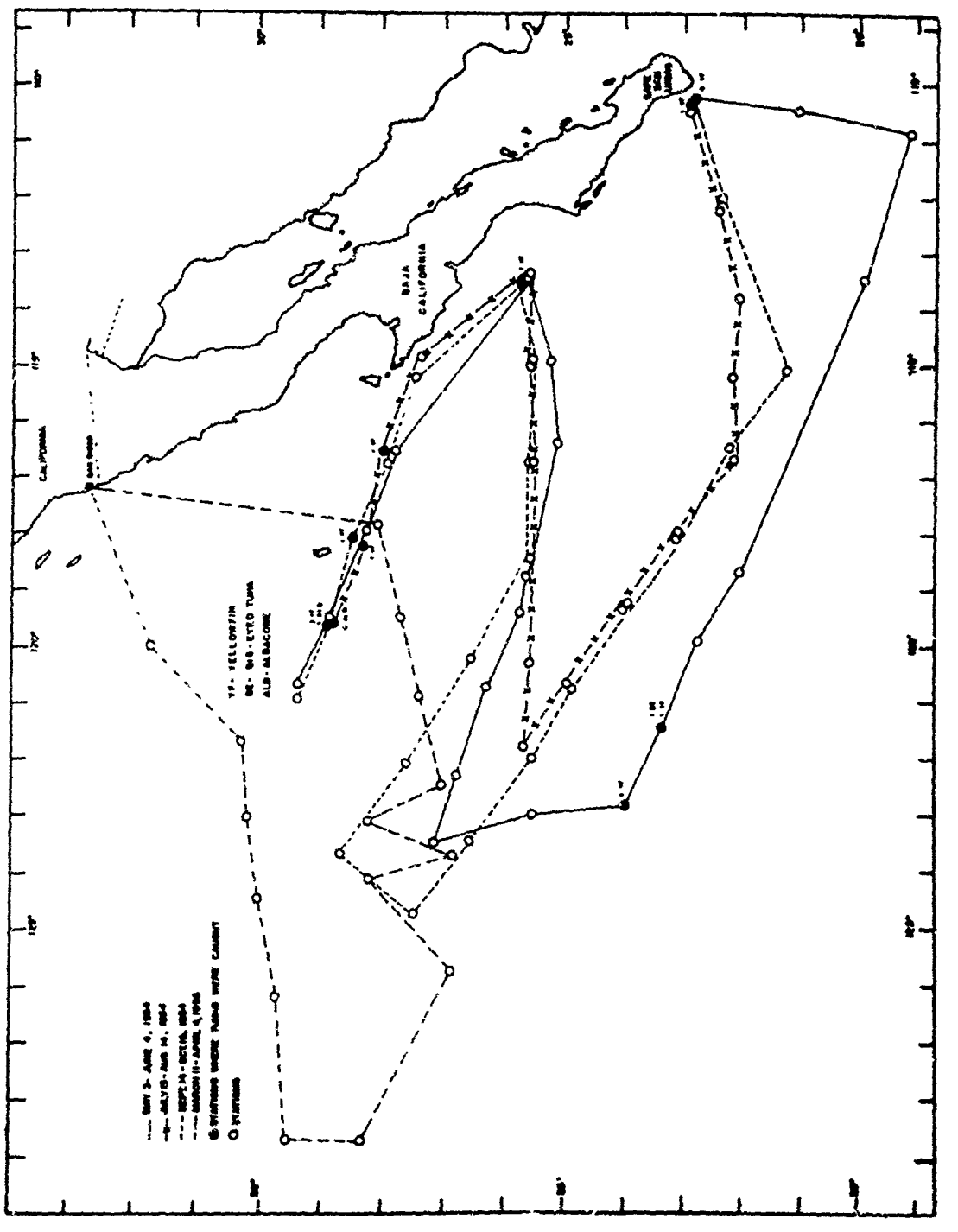


Fig. 6.1—M/V Paolina-T long-line cruises.

Table 6.1—SUMMARY OF PAOLINA-T LONG-LINE FISHING RESULTS

	No. of Sets	No. of 6 Hook Baskets Set	Catch				
			Tunas	Spear-fishes	Sharks	Misc.	Total
Cruise No. 1 (May 3-June 4, 1954)	21	952	7	2	143	15	167
Cruise No. 2 (July 13-Aug. 14, 1954)	18	822	6	38	64	24	132
Cruise No. 3 (Sept. 14-Oct. 19, 1954)	22	1036	7	22	66	20	115
Cruise No. 4 (Mar. 11-Apr. 14, 1955)	15	643	0	0	247	5	252
Total	76	3453	20	62	520	64	666

These results showed that long-line fishing for tunas and other economically important fishes was very unproductive, the over-all catch rate for tunas alone being 0.12 fish per 100 hooks. In areas where commercial long-line fishing for tunas is carried on, a catch of from three to four fish per 100 hooks is regarded as about average. The data also indicated that those tunas which were captured by the long lines were most likely the members of a migratory population rather than a resident population because they were all taken on known fishing banks at a time when commercial fishing was in progress. In further support of this is the fact that during the Mar. 11 to Apr. 4, 1955, cruise, when there was no fishing operation, the long lines did not take a single tuna or spearfish. Generally, therefore, it was concluded that the standing crop of food fish was relatively low in the test area during the warmer months of the year and virtually nil during the early spring months, the northern and offshore areas being poorer than the region to the south and along the coastal shelf.

6.2 TEST AND POSTTEST SURVEY

Long-line fishing for large carnivorous fishes was undertaken by the Paolina-T within the test area on 11 days between May 11 and 24, 1955. Nine of the 11 stations occupied were fished during the posttest period. During this survey, 402 baskets (2412 hooks) of long lines were set, and these caught a total of 15 sharks, one snake mackerel, and one opah for an average catch rate of 0.70 fish (all species) per 100 hooks. No tunas or commercially valuable fishes were taken, nor were any signs of the presence of such populations detected during the survey. Figure 6.2 shows the location of each fishing station with reference to Ground Zero. The post-test stations were selected so that fishing could be conducted as closely to the contaminated water areas as possible within limits of safety.

The failure of the long lines to capture any important food fishes indicated that the possibility of contaminated fishes entering into normal food channels as a direct result of Wigwam was minimal.

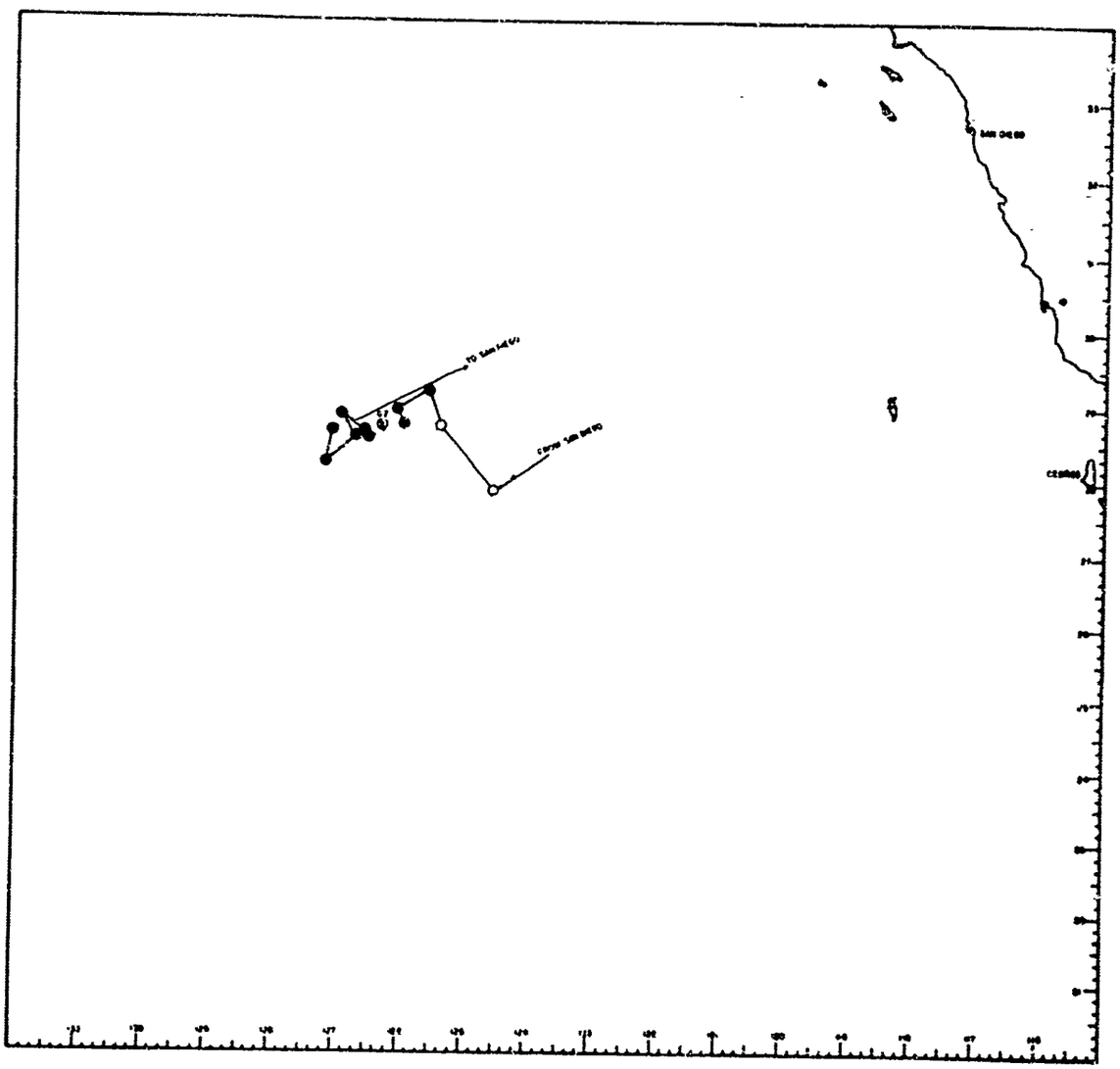


Fig. 6.2—M/V Paolina-T pretest and posttest long-line fishing stations, May 11 to 24, 1955. O, pretest. ●, posttest.

CHAPTER 7

BIOLOGICAL REMOVAL OF RADIOISOTOPES Sr^{90} AND Y^{90} FROM SEA WATER BY MARINE MICROORGANISMS

By Donald W. Lear, Jr., and Carl H. Openheimer, Jr.

7.1 RETENTION OF RADIOISOTOPES BY MARINE MICROORGANISMS

The removal of radioisotopes from a marine environment by microorganisms has implications of immediate interest, with respect both to disposal practices and to military devices.

The measurement of the biological removal of radioisotopes, by both uptake and sorption, by unicellular organisms presents a variety of complicating factors, the chief of which is the extent of the binding power of the cell with the radioisotope. Microorganisms present a large surface to volume ratio; therefore one must discriminate between quantities truly taken into the cell, the quantities firmly held by surface forces, and the quantities but loosely associated. In addition, selective retention of different isotopes from a mixture should be evaluated.

The following experiments were designed to develop accurate and practicable experimental methods for determining microbiological uptake of certain radioisotopes. Many of the experiments were complementary or did not prove reliable; however, they have been included in this report for evaluation. Several approaches to the problem were undertaken, and these approaches are categorized in Secs. 7.1.1 to 7.1.3.

7.1.1 Autoradiography of Bacterial Colonies

Bacteria grown on radioactive media indicate the qualitative concentration of the radioisotope. This procedure will not disclose the nature of the forces involved or lend itself well to quantitative measurement. With the procedures employed, selective uptake of isotopes from mixtures cannot be readily discerned.

7.1.2 Uptake of Radioisotopes from Liquid Media

The separation of cells from the radioactive medium in which they were grown shows the degree to which radioactive materials are bound to the cells. This procedure possibly delineates the materials truly incorporated into the cells (uptake) from those loosely bound by surface forces on the cells (sorbed).

7.1.3 Chemical Fractionation of Cellular Components of Washed Radioactive Cells

This procedure indicates the nature of the binding agencies involved in the true uptake of isotopes.

7.2 EXPERIMENTAL PROCEDURE

Cells of the marine bacterium Serratia marinorubra were used, and their colonies show a characteristic bright red color, which could prove to be a safety feature in the laboratory in the event of accident or catastrophe. In addition, cells of the marine green algal phytoplankter Platymonas subcordiformis were used, and, in a few autoradiography experiments, indigenous bacteria from raw sea-water samples were used.

The isotopes were a carrier-free Sr^{90}-Y^{90} mixture in equilibrium of decay, dated May 13, 1954, and had an activity of 11.96 ± 10 per cent mc/ml. As purchased, this mixture also contained approximately 20 per cent Sr^{89}. For experimental use, a 1:100 dilution in distilled water was used for a working solution. At the time of these experiments the calculated activity of Sr^{89} (assuming six half lives) would have been approximately 1 count/min in the experimental material; consequently it was deemed unnecessary to correct for this isotope.

Sr^{90} and Y^{90} are both beta emitters (no gamma); Sr^{90} has an energy of 0.537 Mev, and Y^{90} has an energy of 2.18 Mev. The half life of Sr^{90} is 25 years, and that of Y^{90} is 2.54 days.

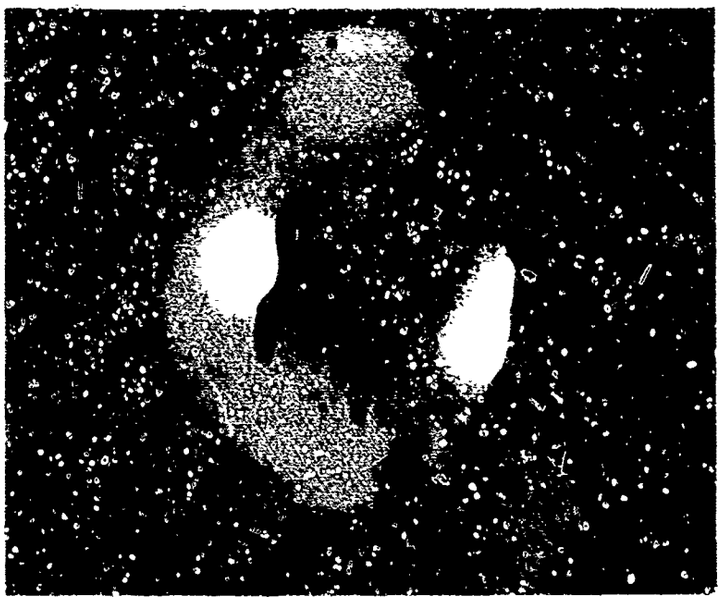
The apparatus used for counting consisted of a Nuclear-Chicago model 182AX scaler connected with a Geiger-Mueller (G-M) tube that had a 1.9 mg/cm^2 mica end window. The calculated efficiency of this apparatus with a calibrated source in position nearest the window was 29.7 per cent.

7.3 AUTORADIOGRAPHY

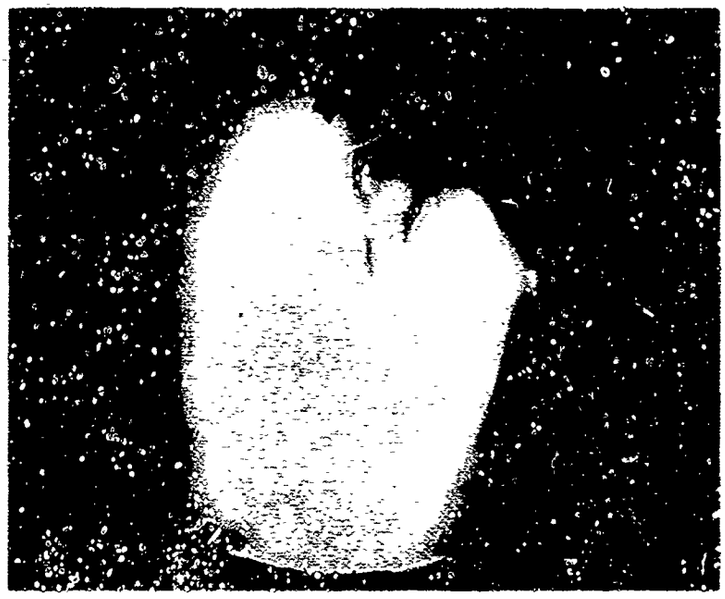
Ten milliliters of sea-water nutrient agar medium, enriched with approximately 1×10^6 dis/min of Sr^{90} and Y^{90}, was poured in petri plates and allowed to solidify. Inocula of Serratia marinorubra, placed at several discrete points on the agar surface with a platinum loop, were allowed to develop to colonies during incubation at 25°C, one series for 48 hr and one for 72 hr. At the end of the incubation period, the colonies were washed off one control plate. The agar, carefully lifted from the petri plates in a darkroom, was placed colony side down against the emulsion of 4 by 5 Eastman metallographic plates. The agar and plates were placed in a lightlight container and stored in a 4°C refrigerator for 48 hr. At the end of this exposure period, the agar was removed, and the plates were developed in DK-50 for 10 min at 22°C. Nonradioactive agar, containing colonies, was used as a control to determine whether media or bacterial cells could interact with the emulsion to produce false results. The controls were all negative.

The results are shown in Fig. 7.1. A repetition of this experiment is shown in Fig. 7.2, except that 5 per cent agar was used in the medium instead of the 2 per cent agar used in the experiment shown in Fig. 7.1. White areas represent colony sites where bacterial concentration of the radioisotopes caused exposure of the emulsion by beta particles. In Fig. 7.2A the concentration of isotopes is present at the colony sites, but the exposure time was not enough to give the contrast of Fig. 7.1. The control plate with radioisotopes but with colonies removed, Fig. 7.2B, indicates that the radioactivity was concentrated in the colonies per se during growth and was then washed off with the colonies.

Colonies cultivated on the surface of millipore filters (molecular filters, MF's) were treated as above. The effective pore size of MF's, 0.5 μ, is capable of retaining bacteria quantitatively on its surface. Raw sea water containing an indigenous bacterial flora was diluted in series with sterile sea water, and aliquots were passed through the MF's. Suspensions of Serratia marinorubra were also used as inocula. Plate counts were made as controls for the dilutions. After filtration the MF's were placed surface up on absorbent pads saturated with 2.0 ml of radioactive sea-water nutrient broth with a calculated activity of approximately 25,000 counts/min per pad. The bacteria were nurtured to colonies by the diffusion of medium up through the MF. Pads and MF's were incubated at 35°C for 48 hr to provide colonies approximately 3 to 6 mm in diameter. The number of colonies was counted. The MF's were removed from the nutrient pads, dried, and placed with the colony side in contact with metallographic plates. After an exposure of 72 hr at 4°C, the plates were developed.

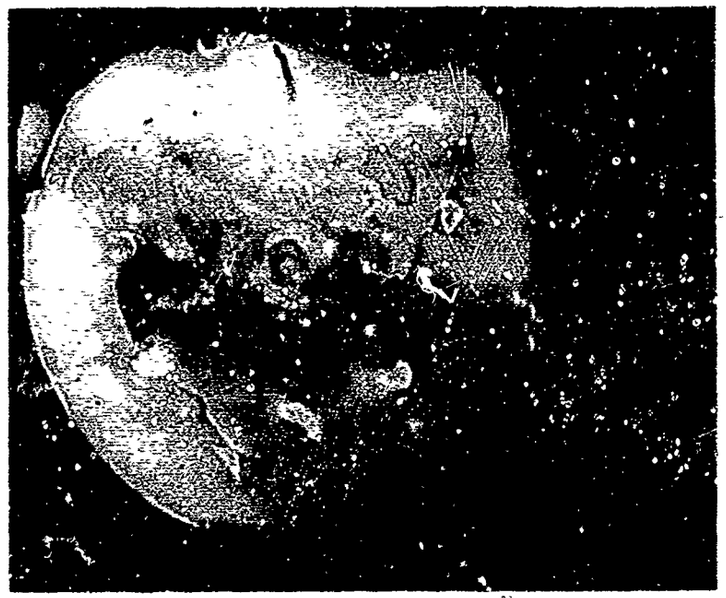


A



B

Fig. 7.1—Autoradiographs of Serratia marinorubra colonies on radioactive sea-water nutrient agar. (The agar surface was directly in contact with the photographic emulsion.) A, 72-hr colonies. B, 72-hr colonies.



A

B

Fig. 7.2—Autoradiographs of Serratia marinorubra colonies on radioactive sea-water nutrient agar. (Tissue was placed between the agar and the photographic emulsion.) A, 48-hr colonies. B, 48-hr colonies, but with the agar washed off.

Control MF's with no colonies were also used. The results are shown in Figs. 7.3 and 7.4, which indicate that the colonies were not able to concentrate the radioisotopes or that the sorption rate of the MF for the radioisotopes was the same throughout the entire surface and interfered with bacterial concentration.

7.4 SEPARATION OF CELLS FROM MEDIUM

To determine the Sr^{90}-Y^{90} uptake quantitatively, bacterial cells grown in radioactive media were separated from the medium by filtering aliquots through MF's. The activity remaining on the MF's was then counted. Sterile radioactive media were used as controls for mechanical sorption and precipitation. Net uptake was calculated as the activity on MF's with cells minus the activity on MF's with no cells.

Six Erlenmeyer flasks were used, each containing 250 ml of sea-water nutrient broth of the following composition: Bacto peptone, 5 g, and sea water, 1000 ml.

A Sr^{90}-Y^{90} working solution with activity approximately 0.1196 mc/ml was added to flasks as follows: flasks 1 and 2, 0.1 ml; flasks 3 and 4, 0.5 ml; and flasks 5 and 6, 1.0 ml. Flasks 1, 3, and 5 were inoculated with a fresh suspension of Serratia marinorubra and incubated at room temperature. At intervals 10-ml aliquots were removed and passed through MF's, dried, and counted. Duplicate series of MF's were washed by passing them through 10 ml of sterile, nonradioactive medium after the aliquot of culture. The noninoculated controls, flasks 2, 4, and 6, were treated in the same manner.

The filters were recounted at successive intervals to follow decay characteristics. The results of this experiment are shown in Figs. 7.5 and 7.6 and Tables 7.1 and 7.2. To eliminate errors of sorption and precipitation from the medium, the uptake values were derived by subtraction of the activity of the MF's of the control flasks from the activity of the MF's from the inoculated flasks.

The values of isotope retention, as percentages, for the bacteria on washed and unwashed filters, Table 7.1, indicate that the efficiency of removal was greater at lower isotope levels in the medium. The time necessary for maximum uptake under these conditions was from four to seven days, as can be seen in the graphical presentation in Fig. 7.5. The negative percentage-retention values found are indicative of precipitation of isotopes from the medium. The lower percentage values of the controls on the fourth day suggest that the precipitation might be affected by fluctuations of temperature since the flasks were incubated at room temperature. None of the control flasks developed perceptible turbidity, eliminating possibilities of contamination. These data suggest that the percentage retention of the isotopes by Serratia marinorubra would range from 6 to 28 per cent.

Table 7.2 and Fig. 7.6 show the decay characteristics of the samples in this experiment. The graph, Fig. 7.6, indicates that an excess of Y^{90}, with respect to Sr^{90}, was taken up by Serratia marinorubra. By calculation it can be estimated that, of the activity retained by the bacteria, at least 82 per cent was due to Y^{90}. Sr^{90} cannot be evaluated because sufficient recounts were not taken to find the leveling of the decay curves, if such occurred. Since the control filters with no cells show the same pattern of decay, it is probable that Y^{90} is precipitating from the medium.

The effect of washing was determined on the retention of radioisotopes by Serratia marinorubra and by the green alga Platymonas subcordiformis when retained on MF's. Serratia marinorubra cells were grown in 250 ml of sea-water nutrient broth containing 0.5 ml of Sr^{90}-Y^{90} working solution. A series of 10-ml aliquots was withdrawn and passed through MF's. The MF's were then washed by passing through them various quantities of sterile nonradioactive medium. The filters were dried and counted.

Platymonas subcordiformis cells (dimensions approximately 5 by 15 μ) were cultivated in a sea-water modification of Beijerinck's medium. A 0.1-ml Sr^{90}-Y^{90} working solution was added to flasks containing 100 ml of medium. The cells were filtered as described above and washed with various quantities of sterile molecular-filtered sea water. The filters were dried

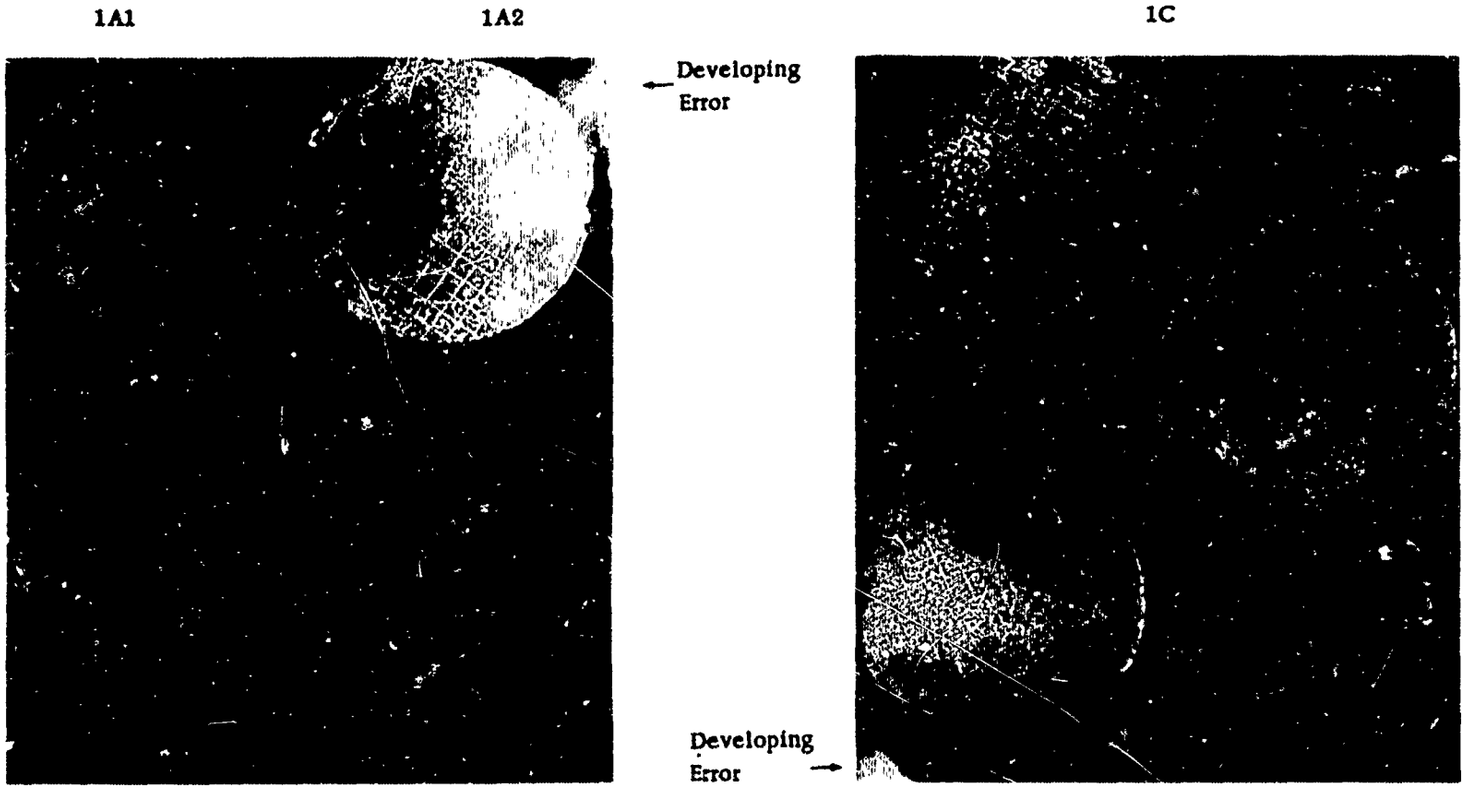


Fig. 7.3—Autoradiography of marine bacteria colonies grown on molecular filters containing radioactive medium. 1A1 and 1A2, seawater sample from Scripps Institution of Oceanography pier, 1:10 dilution, 5.0 ml through filter; conventional agar plate count showed 1 bacterium/ml; observable colonies (27×) on dried filters before autoradiography: 1A1, 25 colonies, and 1A2, seven colonies. 1B1 and 1B2, same sample but diluted 1:100; conventional agar plate count showed 1 bacterium/ml; observable colonies (27×) on dried filters before autoradiography: 1B1, two colonies, and 1B2, one colony.

Fig. 7.4—Autoradiography of marine bacteria colonies grown on molecular filters containing radioactive medium. Sample 1C (upper two filters): Serratia marinorubra suspension in sea water, 5.0 ml through filter; conventional agar plate count showed 1125 bacteria/ml; colonies on dried filters were too numerous to count but were primarily discrete. Controls (lower two filters): filters placed on nutrient pads containing radioactive medium; no colonies were observable.

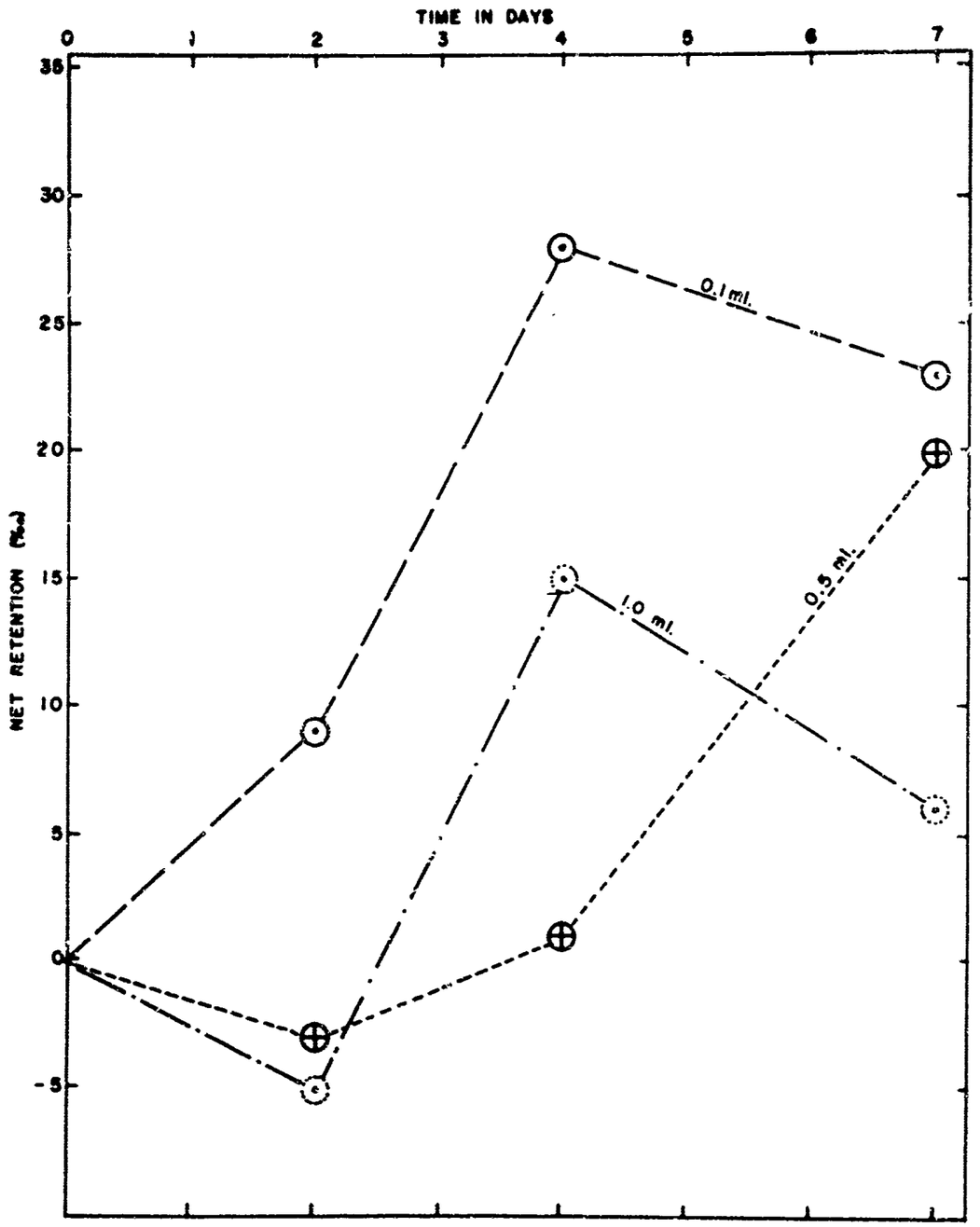


Fig. 7.5—Retention of radioisotopes by Serratia marinorubra (washed filter data).

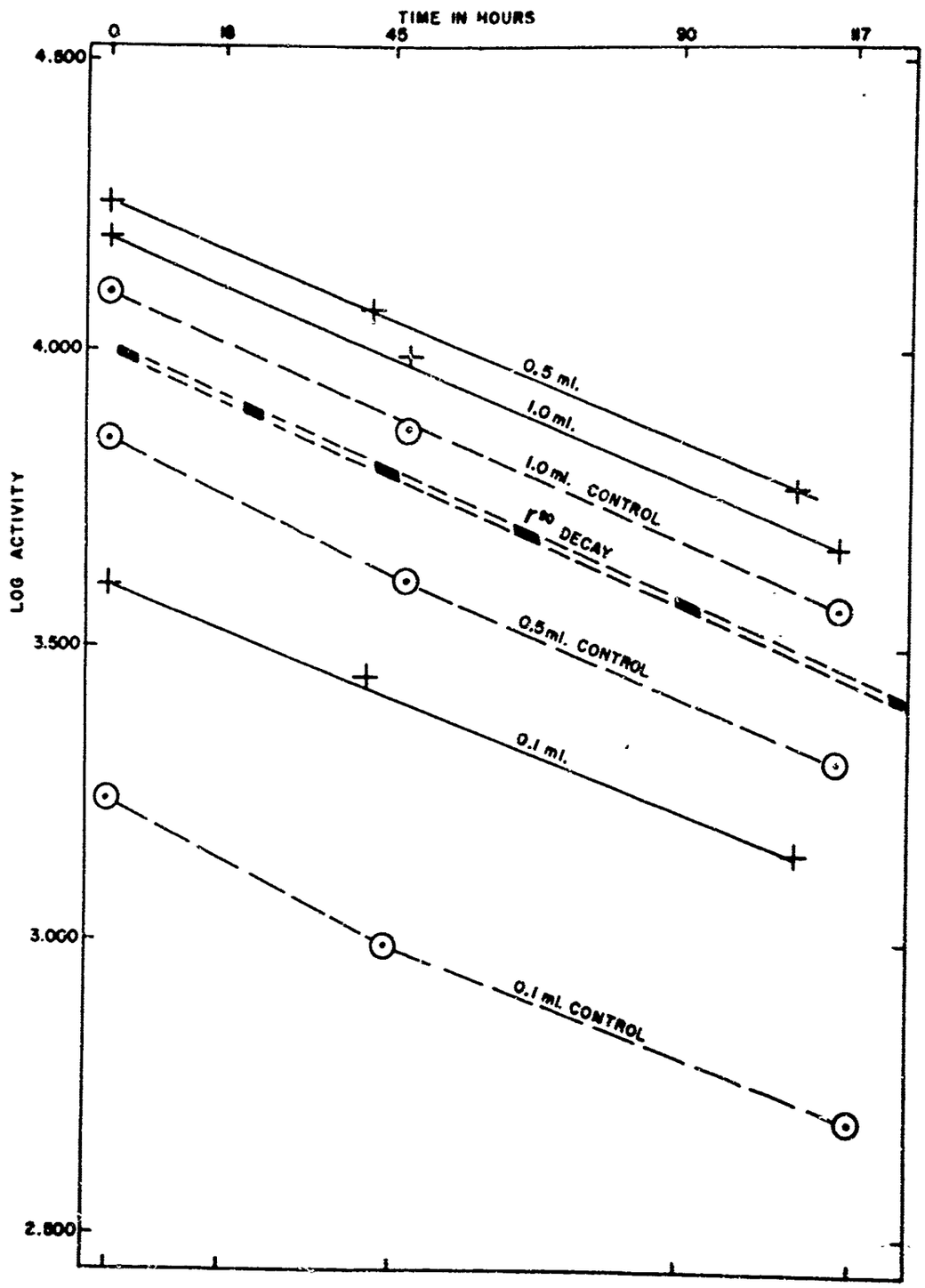


Fig. 7.6—Decay of isotopes retained by Serratia marinorubra (washed filters from seventh day).

Table 7.1 — RETENTION OF RADIOACTIVITY FROM RADIOACTIVE SEA-WATER NUTRIENT BROTH BY THE MARINE BACTERIUM *Serratia marinorubra*

WASHED FILTERS

Flask	Isotope level (ml working solution added)	Initial count cpm/10 ml	Activity of filters, cpm			% Initial count			Retention, % (inoculated - control)		
			2 days	4 days	7 days	2 days	4 days	7 days	2 days	4 days	7 days
1.	0.1 ml Inoculated	12752	2644	4378	4041	21	34	32	9	28	23
2.	" Control	18820	2292	1072	1728	12	6	9			
3.	0.5 ml Inoculated	63940	6406	4798	18629	10	8	29	-3	1	20
4.	" Control	83050	10841	5595	7202	13	7	9			
5.	1.0 ml Inoculated	99830	18290	23909	15879	8	24	16	-5	15	6
6.	" Control	120070	15254	10410	12683	13	9	10			

FILTERS NOT WASHED

Flask	Isotope level	Initial count cpm/10 ml	Activity of filters, cpm				% Initial count				Retention, %			
			1 day	2 days	4 days	7 days	1 day	2 days	4 days	7 days	1 day	2 ds	4 ds	7 ds
1.	0.1 ml Inoculated	12752	2059	2851	*17673	4999	16	22	*139	39	-	10	*130	27
2.	" Control	18820	-	2267	1742	2224	-	12	9	12				
3.	0.15 ml Inoculated	63940	5683	7764	19468	19863	9	12	30	31	-	-1	21	21
4.	" Control	83050	-	10843	7313	8111	-	13	9	10				
5.	1.0 ml Inoculated	99830	6937	8172	26402	30809	7	8	26	31	-3	-5	16	20
6.	" Control	120070	12715	16046	12177	13432	10	13	10	11				

* Probably anomalous sampling.

Table 7.2—DECAY OF ISOTOPES RETAINED BY *Serratia marinorubra*

	Filters from Second Day					Filters from Fourth Day				
	WASHED					**WASHED**				
	Initial cpm	log cpm	Δt hours	cpm	log cpm	Initial cpm	log cpm	Δt hours	cpm	log cpm
0.1 ml Inoculated	2644	3.42226	75½	1201	3.07954	4378	3.64128	47	2862	3.45667
" Control	2292	3.36021	"	957	2.98091	1072	3.03019	"	732	2.86451
0.5 ml Inoculated	6406	3.80659	"	2880	3.45939	4798	3.68106	"	2926	3.46627
" Control	10841	4.03507	"	5004	3.69932	5595	3.74780	"	3802	3.58001
1.0 ml Inoculated	8090	3.91855	"	3798	3.57955	23909	4.37856	"	16272	4.21144
" Control	15254	4.18338	"	6948	3.84186	10410	4.01745	"	7087	3.85046
	NOT WASHED					**NOT WASHED**				
0.1 ml Inoculated	2851	3.45500	75½	1321	3.12090	17673	4.24731	47	11814	4.07240
" Control	2267	3.35545	"	1146	3.05918	1742	3.24105	"	1250	3.09691
0.5 ml Inoculated	7764	3.89009	"	3742	3.57310	19468	4.28932	"	12164	4.08507
" Control	10843	4.03515	"	5124	3.70961	7313	3.86410	"	5223	3.71792
1.0 ml Inoculated	8372	3.91233	"	4162	3.61930	26402	4.42163	"	18086	4.25734
" Control	16046	4.20537	"	7525	3.87651	12177	4.08554	"	8661	3.93857

Filters from Seventh Day

	WASHED											
	Initial cpm	log cpm	Δt hours	cpm	log cpm	Δt hours	cpm	log cpm	Δt hours	cpm	log cpm
0.1 ml Inoculated	4043	3.60649	41½	2793	3.44607	-	-	-	109	1412	3.14983
" Control	1728	3.23754	46	1017	3.00732	-	-	-	117	503	2.70157
0.5 ml Inoculated	18266	4.26172	41½	11805	4.07206	-	-	-	108½	5912	3.77173
" Control	7202	3.85745	48	4125	3.61542	-	-	-	115	2086	3.31931
1.0 ml Inoculated	15879	4.20082	48	9462	3.97598	-	-	-	115	4693	3.67145
" Control	12683	4.10322	46	7432	3.87105	-	-	-	115	3756	3.57473
	NOT WASHED										
0.1 ml Inoculated	4999	3.69888	47½	3136	3.49638	100	1881	3.27439	120½	1544	3.18865
" Control	2224	3.34713	"	1434	3.15655	"	884	2.94645	120	751	2.87564
0.5 ml Inoculated	19869	4.29804	"	12557	4.09888	"	7508	3.87552	120	6332	3.80145
" Control	8111	3.90907	"	5087	3.70646	-	-	-	120½	2649	3.42308
1.0 ml Inoculated	30600	4.48585	48	19085	4.28070	-	-	-	120½	9833	3.99269
" Control	13432	4.12814	49½	8356	3.92200	-	-	-	121½	4504	3.65360

and counted at successive intervals to determine decay characteristics of the isotopes retained by the cells.

The results are shown in Tables 7.3 and 7.4 and Fig. 7.7. The data in Table 7.3 indicate that part of the isotopes were sorbed on cell surfaces. Most of these sorbed isotopes were

Table 7.3—EFFECT OF WASHING ON RETENTION OF ISOTOPES BY Serratia marinorubra AND Platymonas subcordiformis

Ten ml aliquots of a bacterial culture of S. marinorubra filtered through MF's, and the filters washed by filtering through a specified amount of sterile, non-radioactive medium.

	cpm/ml	% of Initial Culture
Initial culture	6294	100
Filter, no wash	1039	16.2
" 25 ml wash	873	13.6
" 50 " "	884	13.8
" 75 " "	887	13.9
" 100 " "	838	13.1

Ten ml aliquots of a bacteria-free culture of the unicellular green alga Platymonas subcordiformis filtered through MF's, and the filters washed by filtering through a specified amount of sterile MF-filtered sea water.

	cpm/ml	% of Initial Culture
Initial culture	3850	100
Filter, no wash	842	21.9
" 10 ml wash	754	19.6
" 25 " "	744	19.3
" 50 " "	726	18.8
" 100 " "	701	18.2

removed by 10 ml of wash. When more than 10 ml (10 to 100 ml) of wash was used, only a small decrease in total activity was noted. The slightly decreasing residual activity of Platymonas subcordiformis cells with more than 10 ml of wash, and of Serratia marinorubra with more than 25 ml of wash, suggests that the isotopes were leached from the cells or that some of the cells were lysed.

The decay characteristics for Platymonas subcordiformis uptake are shown in Table 7.3 and Fig. 7.7. The decay curve for the original medium indicates that the Sr^{90}-Y^{90} equilibrium had been disturbed through the loss of some Y^{90}. The increase of activity is characteristic of the activity of Y^{90} as it builds up to an equilibrium value with the parent isotope Sr^{90}. This loss is probably due to precipitation and sorption of Y^{90} on the glassware. The dashed portions of the decay curves in Fig. 7.7 represent the presumed course of decay for the mixture.

The curves for the MF's show an initial decrease in activity which then levels off, indicating that both Y^{90} and Sr^{90} had been retained. Assuming, on a graph of this time scale, that the decay curve for the Sr^{90}-Y^{90} equilibrium is a horizontal line, the relative quantities of isotopes can be calculated. Comparing the selection of isotopes by Platymonas subcordiformis and Serratia marinorubra, it can be calculated from data in Table 7.4 that, of the activity retained by Platymonas subcordiformis, 40.8 per cent was due to Sr^{90} and 59.2 per cent was due to Y^{90}. A similar calculation for Serratia marinorubra from data in Table 7.8 shows that 95.7 per cent of the activity retained was due to Y^{90} and 4.3 per cent was due to Sr^{90}.

Table 7.4—DECAY CHARACTERISTICS OF Platymonas subcordiformis EXPERIMENT

	cpm	log cpm	Δt	cpm	log cpm	Δt	cpm	log cpm	Δt	cpm	log cpm
Initial culture	2011	3.30341	45 d	2142	3.33082	203 d	2097	3.32160	232 d	2017	3.30471
" "	1839	3.26456	"	1950	3.29003	"	1960	3.29226	"	1866	3.27091
Filter, no wash	8369	3.92267	"	7217	3.85836	"	6758	3.82982	"	6588	3.81875
" "	8469	3.92783	"	7506	3.87541	"	7055	3.84850	"	6847	3.83550
Filter, 10 ml wash	7653	3.88383	"	6621	3.82092	"	6284	3.79824	"	6111	3.78611
" "	7436	3.87134	48 d	5916	3.77203	"	6002	3.77830	"	5958	3.77510
Filter, 25 ml wash	7482	3.87408	"	6100	3.78533	"	6179	3.79092	"	6092	3.78476
" "	7399	3.86917	"	6027	3.78010	"	6193	3.79190	"	6002	3.77830
Filter, 50 ml wash	7206	3.85769	"	5949	3.77444	"	6115	3.78640	"	5921	3.77240
" "	7310	3.86392	"	6043	3.78125	"	6066	3.78290	"	5953	3.77474
Filter 100 ml wash	6937	3.84117	"	5963	3.77546	"	6045	3.78140	"	5898	3.77070
" "	7089	3.85058	"	6001	3.77822	"	6036	3.78075	"	5948	3.77437

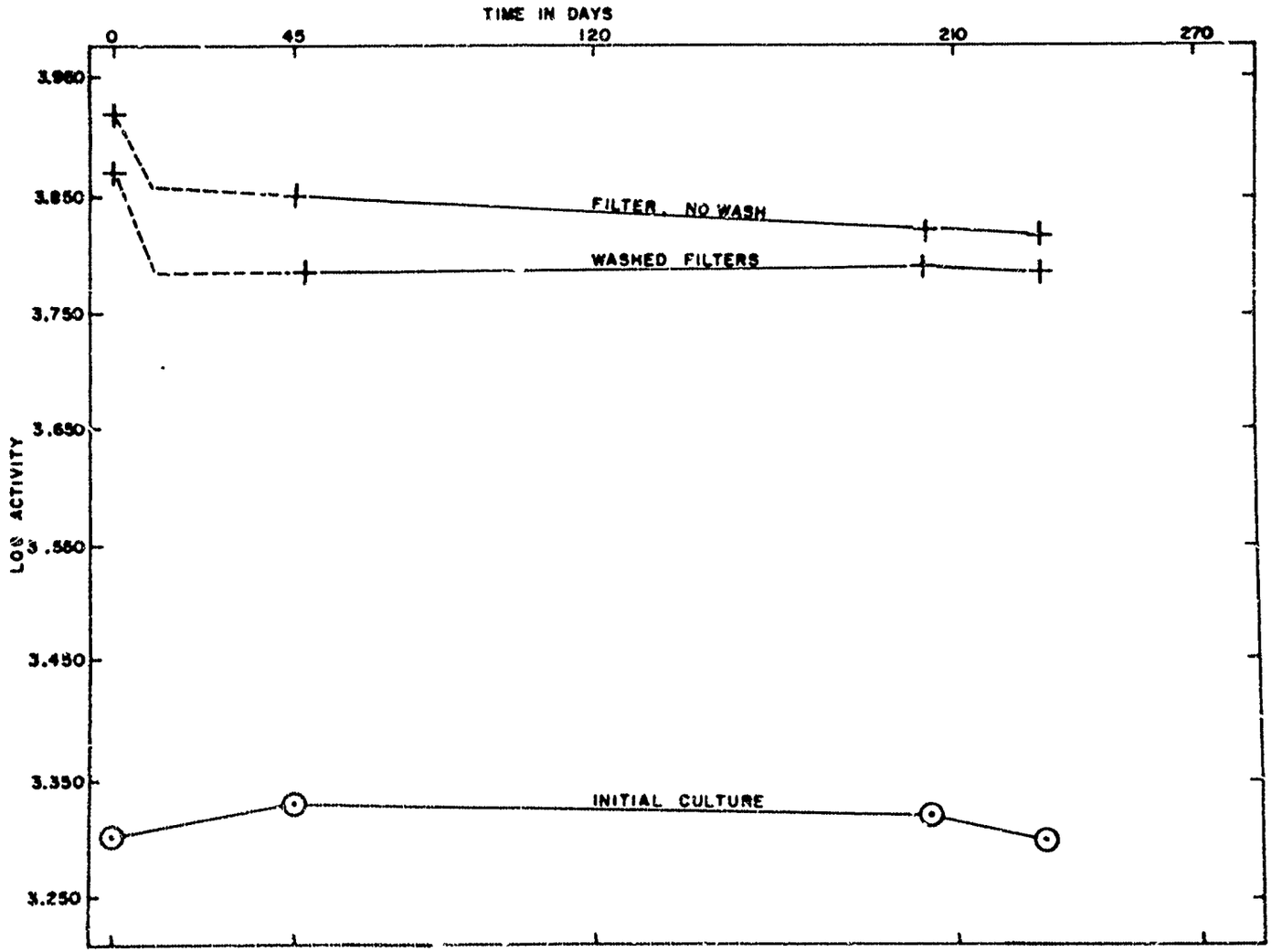


Fig. 7.7—Decay characteristics of the Platymonas subcordiformis experiment.

The results of a modification of the filtration technique are shown in Tables 7.5 and 7.6 and in Fig. 7.6. In this experiment Serratia marinorubra cells were grown in radioactive sea-water nutrient broth (approximately 0.0598 mc of Sr^{90} and Y^{90} in 250 ml) at 25°C. The cells were harvested by centrifugation and resuspended in 100 ml of sterile nonradioactive sea water. The suspended cells were placed in a dialysis membrane and dialyzed against 200 ml of distilled water for five days. Samples of dialyzed cells and dialysate were then plated for counting. The dialyzed cells were also examined microscopically. Many were actively motile, and no abnormally shaped forms were seen. Aliquots of 10 ml were withdrawn and passed through MF's. Replicate series of MF's were washed with various quantities of molecular-filtered sea water. The filters were dried and counted at successive intervals.

Table 7.5—EFFECT OF WASHING DIALYZED Serratia marinorubra CELLS

	Volume	Total Activity	% of activity of cells	Calculated $\%Sr^{90}$	Calculated $\%Y^{90}$
Dialyzed cells in suspension	100 ml	137,900 cpm	100	12.3	87.7
Dialysate	200 ml	53,360	38.7	50	50
Filter, no wash	(10 ml)	12,180	88.3	0.95	99.05
Filter, 50 ml wash	(10 ml)	6,580	47.7	0.68	99.32
Filter, 100 ml wash	(10 ml)	11,300	81.9	0.71	99.29

Note: In this experiment, 100 ml wash required 18 hours to pass through filter, and 50 ml wash required 8 hours.

Table 7.6—EFFECT OF WASHING DIALYZED Serratia marinorubra CELLS—DECAY CHARACTERISTICS

	cpm	log cpm	Δt	cpm	log cpm	Δt	cpm	log cpm
Dialyzed cells	1361	3.13386	154 d	354	2.54900	180 d	314	2.49693
	1396	3.14489	"	340	2.53148	"	345	2.53782
Dialysate	1256	3.09899	"	1461	3.16465	"	1379	3.13956
	1412	3.14983	"	1580	3.19866	"	1344	3.12840
Filter, no wash	12120	4.08350	"	239	2.37845	"	231	2.36361
	12232	4.08750	"	232	2.36549	"	227	2.35603
Filter, 50 ml wash	5412	3.73336	"	74	1.86923	"	63	1.79934
	7733	3.88835	"	110	2.04139	"	111	2.04532
Filter, 100 ml wash	10131	4.00521	"	171	2.23300	"	156	2.19312
	12434	4.09391	"	163	2.21219	"	148	2.17026

As can be seen in Table 7.5, the percentage retention was appreciably higher than the retention shown in Table 7.1. It must be pointed out, however, that 50 ml of wash required 8 hr to filter and 100 ml of wash required 18 hr to filter. These data indicate that the retained isotopes were relatively firmly bound within the cells, since only approximately 26 per cent of the activity was lost by dialysis against distilled water after five days and since the percentage retention on the filters was relatively greater. The effects of washing in this case must be viewed with caution because of the abnormally long time necessary for filtering wash water through. This probably contributes to the erratic percentage values obtained.

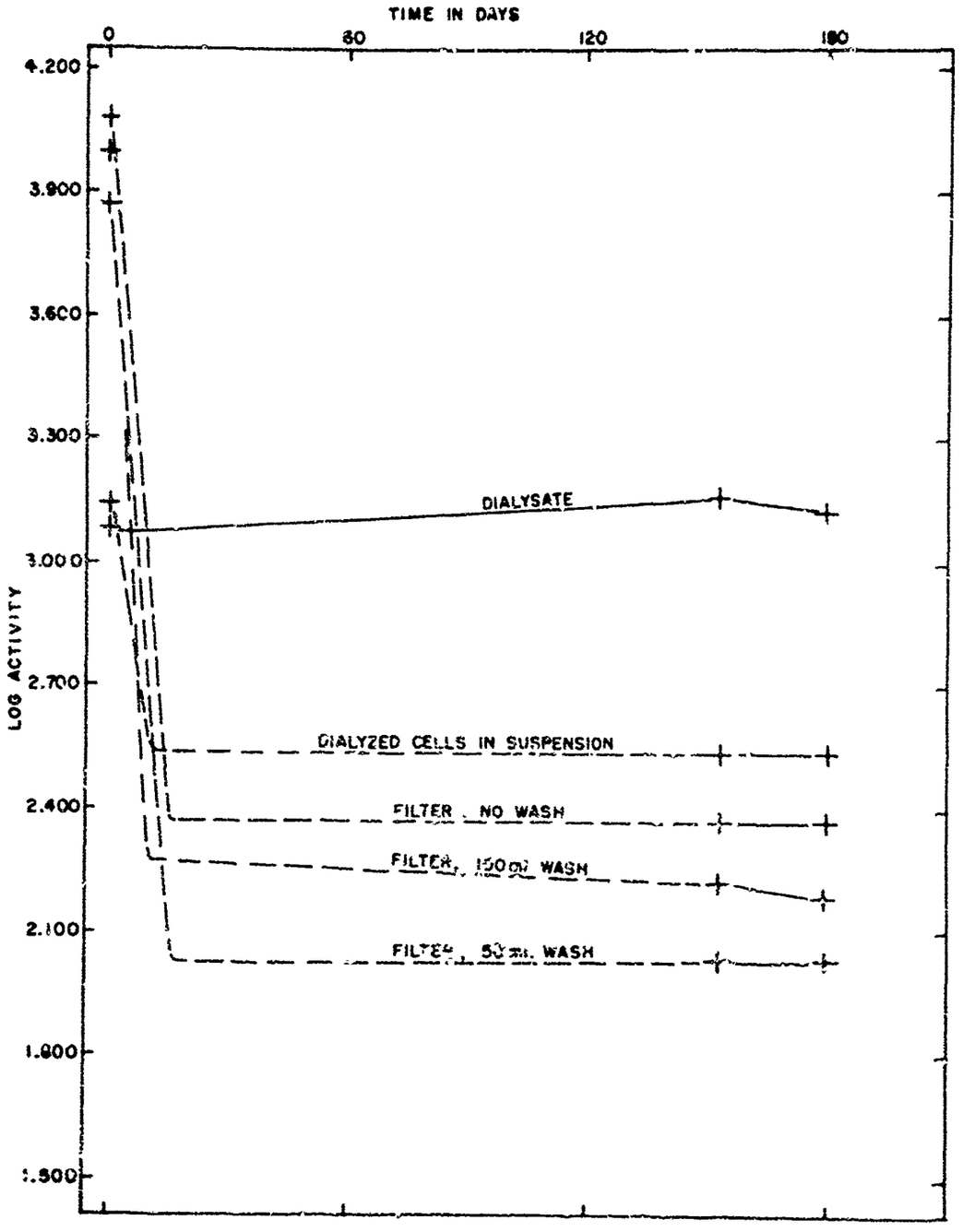


Fig. 7.8—Effect of washing dialyzed Serratia marinorubra cells (decay characteristics).

The decay characteristics are shown in Table 7.6 and Fig. 7.8. As can be seen on the graph, Fig. 7.8, the dialysate showed a slight deficiency of Y^{90}, whereas the cells showed an excess of Y^{90}. The decay curve of the filters indicates that the bacterial cells retained a great excess of Y^{90} as compared to Sr^{90}. These amounts were calculated and are shown in Table 7.5. Again it can be noted that Serratia marinorubra cells selectively bind Y^{90}, with nearly the exclusion of Sr^{90}, when calculated on an activity basis.

In a further experiment on uptake, dilute suspensions of Serratia marinorubra were filtered through MF's, and each filter was then placed carefully upon a pad saturated with radioactive sea-water nutrient broth. The bacteria on the filter surface were nourished by diffusion of medium up through the filter, and colonies formed. Of six filters treated in this way, two were dried without washing, two were replaced in the filtration apparatus and washed with 25 ml of sterile sea water, and two were washed with 100 ml of sterile sea water. The filters were dried and counted.

The results are shown in Table 7.7. It is interesting to note the similar order of magnitude of uptake as compared with the filtration experiment in Table 7.1, but insufficient controls were made for valid calculations.

Table 7.7—WASHING OF CELLS GROWN ON MOLECULAR FILTERS

	cpm/filter	% of value of filter with no wash
Filter, no wash	25,223	100
Filter, 25 ml wash	2,959	11.7
Filter, 100 ml wash	4,634	18.4

Another modification of the separation procedure on MF's was undertaken in which MF's with radioactive bacteria collected on their surfaces, by filtration, were digested in a sulfuric acid and perchloric acid mixture. Only the mineral content remained after digestion; this was then diluted, and aliquots were counted. Quite variable results were obtained, and the procedure proved to be hazardous and time consuming. However, this procedure would improve counting geometry by virtue of using planchets rather than counting filters directly.

An experiment was undertaken to evaluate the effect of cell surface forces in binding isotopes. A culture of Serratia marinorubra cells was killed and preserved by adding 10 per cent neutral Formalin, 1:1; and the cells, which were centrifuged, resuspended in sterile sea-water nutrient broth, and examined microscopically, appeared intact and numerous. Aliquots of the suspension were filtered as in the above-mentioned experiments. No conclusions could be drawn, for uninoculated-medium controls showed more activity on the filters than samples with organisms, presumably owing to precipitation of isotopes from the medium.

In one experiment, attempts were made to evaluate the effect of pH on the retention of isotopes and also to compare the filtration and centrifugation methods for separating cells from media.

Serratia marinorubra cells were cultivated in sea-water nutrient broth containing Sr^{90} and Y^{90}. After incubation the culture was divided into three flasks and adjusted to pH 8.3, pH 7.0, and pH 6.0, respectively (the incubated culture was 8.3, so no adjustment was necessary). Aliquots of 5 ml were withdrawn from each flask and filtered through MF's, which were dried and counted. Other aliquots from the flasks were withdrawn and centrifuged, and the supernatants were plated on planchets for counting. Centrifugates were resuspended at their appropriate pH values and counted, and aliquots were filtered through MF's. The results of this experiment are shown in Tables 7.8 and 7.9 and Fig. 7.9.

Owing to a laboratory accident, accurate sampling of the activity of the original medium was not obtained. Consequently the percentages in Table 7.8 were calculated on the basis of

Table 7.8—SEPARATION AT DIFFERENT pH VALUES OF *Serratia marinorubra* CELLS FROM RADIOACTIVE MEDIUM BY FILTRATION AND CENTRIFUGATION

Filtration Method

pH	Total Activity of Filter, cpm	$\%Y^{90}$	$\%Sr^{90}$	Total Activity of Filtrate, cpm	$\%Y^{90}$	$\%Sr^{90}$	% Total Activity Retained on Filter	% Total Activity Passed Through Filter
8.3	154778	95.8	4.2	245016	34.3	65.7	38.7	61.3
7.0	184796	96.5	3.5	206536	25.2	74.8	47.1	52.9
6.0	234362	97.0	3.0	186928	14.5	85.5	55.6	44.4

Centrifugation Method

pH	Total Activity of Supernatant, cpm	$\%Y^{90}$	$\%Sr^{90}$	Total Activity of Centrifugate, cpm	$\%Y^{90}$	$\%Sr^{90}$	% Total Activity in Supernatant	% Total Activity in Centrifugate
8.3	686057	38.3	61.2	133950	95.9	4.1	83.7	16.3
7.0	647733	36.9	63.1	221382	92.3	7.7	74.5	25.5
6.0	464287	14.7	85.3	230760	97.9	2.1	66.8	33.2

Filtration of Resuspended Centrifugate

pH	Total Activity of Suspension	$\%Y^{90}$	$\%Sr^{90}$	Total Activity of Filter	$\%Y^{90}$	$\%Sr^{90}$	Total Activity of Filtrate	$\%Y^{90}$	$\%Sr^{90}$	% Total Activity on Filter	% Total Activity in Filtrate
8.3	133950	95.9	4.1	69360	99.74	0.26	7230	28.6	71.4	51.8	5.4
7.0	221385	92.3	7.7	125320	99.73	0.27	21510	16.6	83.4	56.6	9.7
6.0	230760	97.9	2.1	154966	99.92	0.08	7005	37.6	62.4	67.2	3.0

Table 7.9—SEPARATION OF CELLS FROM MEDIUM AT DIFFERENT pH VALUES—DECAY CHARACTERISTICS

	cpm	log cpm	Δt	cpm	log cpm	Δt	cpm	log cpm	Δt	cpm	log cpm
Original	35136	4.54575	17 d	37006	4.56827	234 d	33007	4.51861	265 d	32042	4.50572
Filter, pH 8.3	154776	5.18967	-	-	-	"	13316	4.12437	"	13029	4.11491
" " 7.0	184800	5.26670	-	-	-	"	13026	4.11471	"	12641	4.10178
" " 6.0	234360	5.36988	-	-	-	"	14171	4.15140	"	13737	4.13789
Filtrate pH 8.3	24502	4.38920	17 d	35853	4.55453	"	32449	4.51120	"	31911	4.50394
" " 7.0	20654	4.31500	"	35966	4.55989	"	31379	4.49664	"	30459	4.48372
" " 6.0	18693	4.27168	"	36257	4.55939	"	32478	4.51159	"	31481	4.49804
Supernatant 8.3	26387	4.42139	16 d	36365	4.56068	233 d	32855	4.51660	264 d	31774	4.50207
" 7.0	24913	4.39641	"	36351	4.56051	"	32158	4.50729	"	30732	4.48759
" 6.0	17857	4.25181	"	34756	4.54103	"	30868	4.48951	"	30053	4.47788
Centrifugate 8.3	4465	3.64982	"	530	2.72428	"	374	2.57287	"	363	2.55991
" 7.0	7379	3.86800	"	1458	3.16376	"	1159	3.06408	"	1120	3.04922
" 6.0	7692	3.88604	"	506	2.70415	"	342	2.53403	"	306	2.48572
Filter from Centrifugate 8.3	69360	4.84111	-	-	-	"	370	2.56820	"	356	2.55145
" 7.0	123972	5.09332	-	-	-	"	392	2.84011	"	640	2.80618
" 6.0	125316	5.09800	-	-	-	"	217	2.33646	"	208	2.31806
Filtrate from Centrifugate 8.3	241	2.38202	16 d	404	2.60638	"	350	2.54407	"	336	2.52634
" 7.0	717	2.85552	"	1318	3.11992	"	1200	3.07918	"	1194	3.07700
" 6.0	234	2.36922	"	343	2.53529	"	292	2.46538	"	294	2.46835

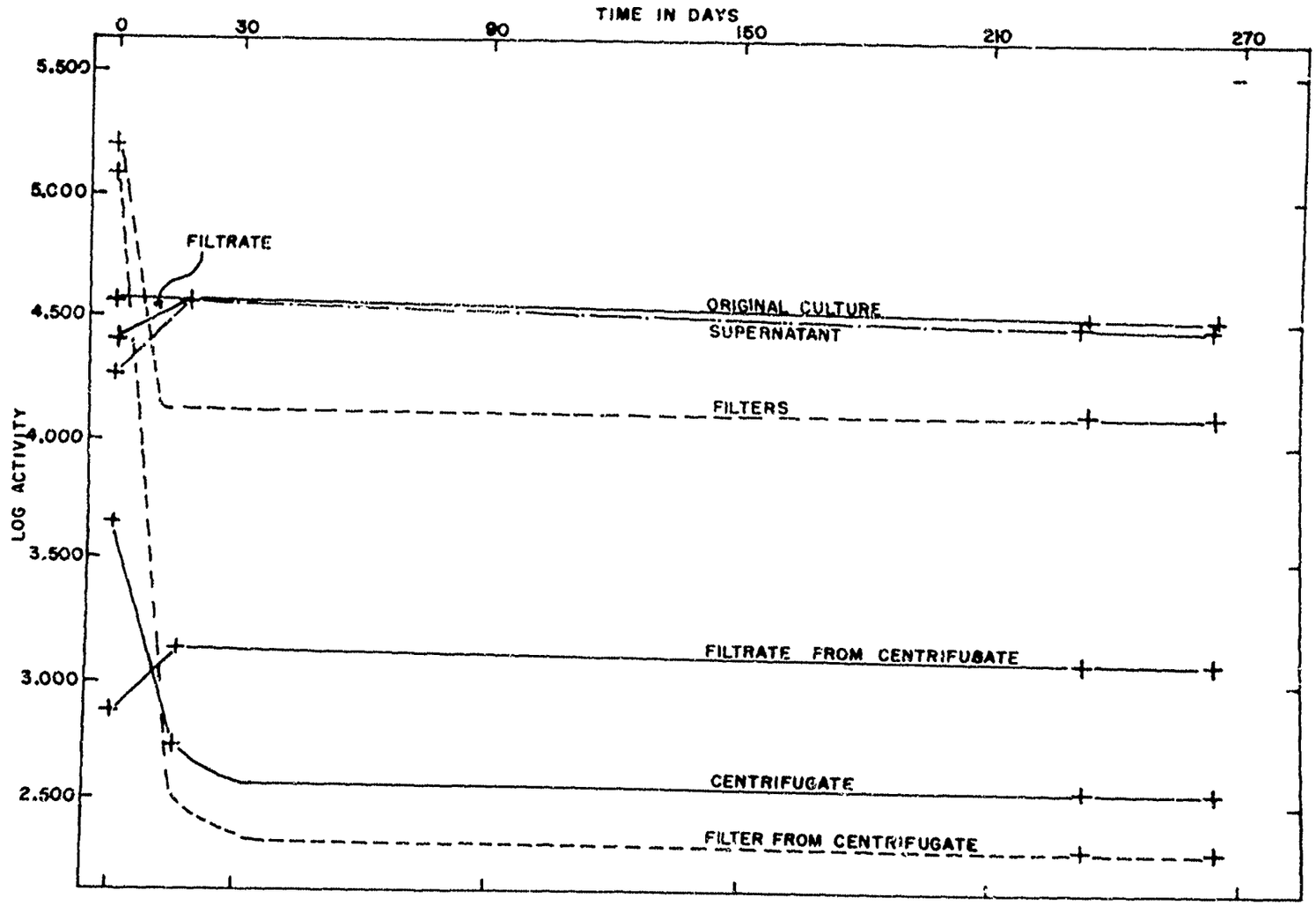


Fig. 7.9—Separation of cells from medium at different pH values (decay characteristics).

the combined activities of filters and filtrate and of the combined activities of supernatant and centrifugate. Total activity is available for data on the filtration of the resuspended centrifugate, and here it can be seen that 30 to 42 per cent of the activity is lost in the filtration process at this level of activity. In the original filtration process, therefore, the percentage passed through the filter is probably too low, which would in turn make the percentage activity of the filters considerably lower.

A comparison of the activity retained on the filters and in the centrifugates at the three pH values would not be affected by these considerations, since all aliquots were from the same original culture. More isotope was retained by the cells at lower pH values, by both centrifugation and filtration.

A comparison of filtration and centrifugation indicates that the latter technique is less effective in separating cells from medium, as evidenced by the 52 to 67 per cent retention when the centrifugate was filtered. This indicates that a considerable amount of unbound isotope is carried down in the centrifugate.

The decay characteristics of the samples in this experiment are shown in Table 7.9 and Fig. 7.9. The original culture shows the characteristic decay of Sr^{90} and Y^{90} in equilibrium. The filtrate and supernatant show a Y^{90} deficiency. The original filters and the centrifugate show an excess of Y^{90}. The filter from the centrifugate shows this excess of Y^{90} also, whereas the filtrate shows a deficiency of Y^{90}.

The relative activities of Y^{90} and Sr^{90} for these samples were calculated from data in Table 7.9 and are shown in Table 7.8. There is no clearly defined difference between the selection of isotope by the cells and the pH of the medium, although there is a tendency for a greater selection of Y^{90} by the cells on the filters at decreasing pH values. This is reflected as well by the lesser percentages of Y^{90} in the filtrates at lower pH values.

In the foregoing experiments it became obvious that radioisotopes were being retained on control filters from media containing no bacteria. Table 7.10 shows the results of an experi-

Table 7.10—RETENTION OF ISOTOPES FROM STERILE MOLECULAR-FILTERED SEA-WATER NUTRIENT BROTH ON MOLECULAR FILTERS

	Activity	Total Activity	% of Total Original Activity
Original medium	4056 cpm/ml	1,104,000 cpm/250 ml	100
First filter	4045 cpm	-	0.40
Second filter (Medium autoclaved)	3155 cpm	-	0.31
Third filter	22,586 cpm	-	2.23
Fourth filter	1505 cpm	-	0.15
Activity of medium	2380 cpm/ml	595,000 cpm/250 ml	58.7
Fifth filter	2211 cpm	-	0.22
Sixth filter	1194 cpm	-	0.12

ment in which 250 ml of sterile radioactive sea-water nutrient broth was passed through two MF's successively. The medium was then autoclaved and passed successively through a series of four MF's. All filters were dried and counted. The results indicate that the filters retained small percentages of the total activity on the first two filters. After autoclaving, the percentage retained was relatively greater, 2.23 per cent, and the next sequential filter was down to 0.15 per cent. Of special interest in this experiment is the fact that 40 per cent of the activity of the medium, as measured by sampling the medium, was lost by forces other than retention in

or on MF's. The filtration apparatus required for MF's involves a sintered-glass base upon which the filters rest, and it is presumed that this 40 per cent was lost primarily by sorption on the sintered-glass bases and secondarily on the walls of the glassware. This conclusion is based upon laboratory experience in decontaminating the filtration apparatus after use. This experiment does indicate a nearly negligible amount of radioactivity retained by the filters per se at this level of activity.

A further experiment was devised to determine whether peptone in the nutrient sea-water broth had appreciable effect on precipitation or the retention of radioactivity on the MF's. Molecular-filtered and unfiltered sea water and filtered and unfiltered sea-water nutrient broth (containing peptone) were placed in flasks. A 0.1-ml Sr^{90}-Y^{90} working solution was added to each flask, and the contents were compared by both filtration and centrifugation techniques. The pH was determined in all flasks. The results shown in Table 7.11 for MF's and centrifugation indicate that the prefiltered solutions lost less activity than those unfiltered. Centrifugation removed more activity in the centrifugate than was removed by the MF's. Precipitation was generally less when prefiltered solutions were used.

The sea water lost less activity than the peptone solution, suggesting that peptone is a factor in precipitation. The pH of sea water did not change as much after autoclaving as the nutrient broth, which might be a factor in the difference in precipitation.

Autoclaving nearly doubled, on the average, the retention of isotopes on the filters. Autoclaving increased retention more in unfiltered than in prefiltered solutions.

The activity lost by sorption to glassware, etc., was greater in filtered than in unfiltered solutions, as indicated by the percentage "lost elsewhere" after autoclaving.

The percentage lost on the filters was greater than in the previous experiment (Table 7.10), probably owing to the lower level of activity in this experiment.

As a result of these experiments a more defined, peptone-free medium was devised for growing Serratia marinorubra. Satisfactory growth was obtained by use of the following medium: glycine, 0.5 per cent; glucose, 1.0 per cent; NH_4NO_3, 0.1 per cent; and sea water.

Preferential extraction of the fractions or components of the bacterial cell that are active in uptake of these isotopes was investigated in a series of experiments. This method, using washed cells, would also indicate whether a true incorporation of isotopes into the cells actually occurred.

In the first experiment Serratia marinorubra cells were grown in 1 liter of sea-water nutrient broth with approximately 0.0598 mc of Sr^{90} and Y^{90} added. The cells were harvested by centrifugation, washed with 100 ml of sterile sea water, and resuspended in 250 ml of sterile molecular-filtered sea water. The bacterial population was estimated turbidimetrically to be 5,800,000 cells/ml Several 10-ml aliquots of this suspension were made.

One 10-ml aliquot of cell suspension was shaken in a separatory funnel with 100 ml of petroleum ether for 4½ hr, and 1 ml of each fraction was plated on planchets, in duplicate, for counting. Separate aliquots were treated with 100 ml of carbon tetrachloride and with benzene as solvents.

Other aliquots were taken, dried under infrared, resuspended with 25 ml of solvent, and centrifuged at 5000 rpm for 5 min. The supernatant and residue were plated in duplicate. Solvents used in this procedure were acetone, distilled water, ethanol, and boiling methanol.

The results of this experiment are shown in Table 7.12. From these results it would appear that the components active in retention are not soluble in the organic solvents screened. Some activity was present in the distilled-water fraction, as would be expected. Decay characteristics of this experiment indicate that all, or nearly all, the activity was due to Y^{90}.

In a second extraction experiment 35 ml of a suspension of Serratia marinorubra cells from the dialysis experiment (compare Table 7.5) was dried under infrared and was then extracted with boiling methanol for 15 min. The resultant solution was centrifuged, and the supernatant and centrifugate were plated. As can be seen in Table 7.13, negligible activity was solubilized by boiling methanol.

A more elaborate extraction scheme was adapted from that of Porter and Knauss.[5] Serratia marinorubra cells were grown in sea-water nutrient broth (0.2392 mc of Sr^{90} and Y^{90}

Table 7.11 — LOSS OF ISOTOPES FROM STERILE SEA-WATER NUTRIENT BROTH AND FROM SEA WATER

Before Autoclaving

	pH	Activity cpm/ml	Total Activity cpm/5 ml	Centrifugation: Supernatant cpm/ml	Centrifugation: % Reduced in Supernatant	Filtration: Activity of Filter, cpm	Filtration: % Total Activity
Sea Water Nutrient Broth	7.7	1847	9235	1693	8.3	477	5.2
Sea Water Nutrient Broth Filtered	7.7	1963	9813	1794	8.6	369	3.8
Sea Water	8.2	1804	9020	1720	5.2	409	4.5
Sea Water Filtered	8.3	1759	8796	1710	2.8	359	4.1

After Autoclaving

	pH	Activity of Filter, cpm	% of Total Original Activity	Activity of Filtrate, cpm/ml	% Original Activity	% Lost Elsewhere
Sea Water Nutrient Broth	8.0	1335	14.4	1153	62.4	18.0
Sea Water Nutrient Broth Filtered	8.0	734	7.5	1917	67.1	21.6
Sea Water	8.3	672	7.4	1192	66.1	22.0
Sea Water Filtered	8.6	550	6.2	1031	58.6	33.1

Table 7.12 — EXTRACTION TO FIND RADIOACTIVE COMPONENTS OF RADIOACTIVE Serratia marinorubra CELLS GROWN IN RADIOACTIVE MEDIUM

Solvent	Fraction	cpm/ml
Petroleum ether	Soluble	?
" "	Insoluble	243
Acetone	Soluble	3
"	Insoluble	9
Distilled Water	Soluble	9
" "	Insoluble	67
Carbon Tetrachloride	Soluble	0
" "	Insoluble	182
Ethanol	Soluble	0
"	Insoluble	155
Benzene	Soluble	0
"	Insoluble	203
Boiling Methanol	Soluble	0
" "	Insoluble	88

Activity of the original medium in which they were grown was 9314 cpm/ml, and the washed cell suspension contained 305 cpm/ml.

Note: Background variation with the counting apparatus used ranges about 6 cpm, consequently counts in this range are of questionable validity.

Table 7.13 — EXTRACTION OF DIALYZED RADIOACTIVE Serratia marinorubra CELLS WITH BOILING METHANOL

	cpm/ml	Total ml	Total Activity, cpm
Cell Suspension	1379	35	48,265
Boiling Methanol Slurry	1869	25	46,725
Centrifugation:			
Supernatant	5	22	110
Centrifugate	15,331	3	45,993

per liter), which was centrifuged and then washed twice. Extraction was done according to the diagrammatic scheme shown in Fig. 7.10. The cells were extracted for 20 min in boiling methanol and centrifuged, and platings were made of the supernatant. The centrifugate was resuspended in 50 ml of 1M NaCl and sampled for counting. The suspension was shaken for 120 min and centrifuged, and the supernatant was plated. The residue was resuspended in 25 ml of a pepsin solution (Braun, USP, lot No. 5553); then it was sampled for counting and allowed to digest at 25°C for 30 min at pH 4.0. The solution was centrifuged, the supernatant was plated, and the residue was washed twice with samplings made of the wash water. The residue was resuspended in 50 ml of 1 per cent HCl; then it was sampled, heated for 30 min at 85°C, and centrifuged, and the supernatant was counted. The residue was slurried in 20 ml of distilled water and was plated for counting.

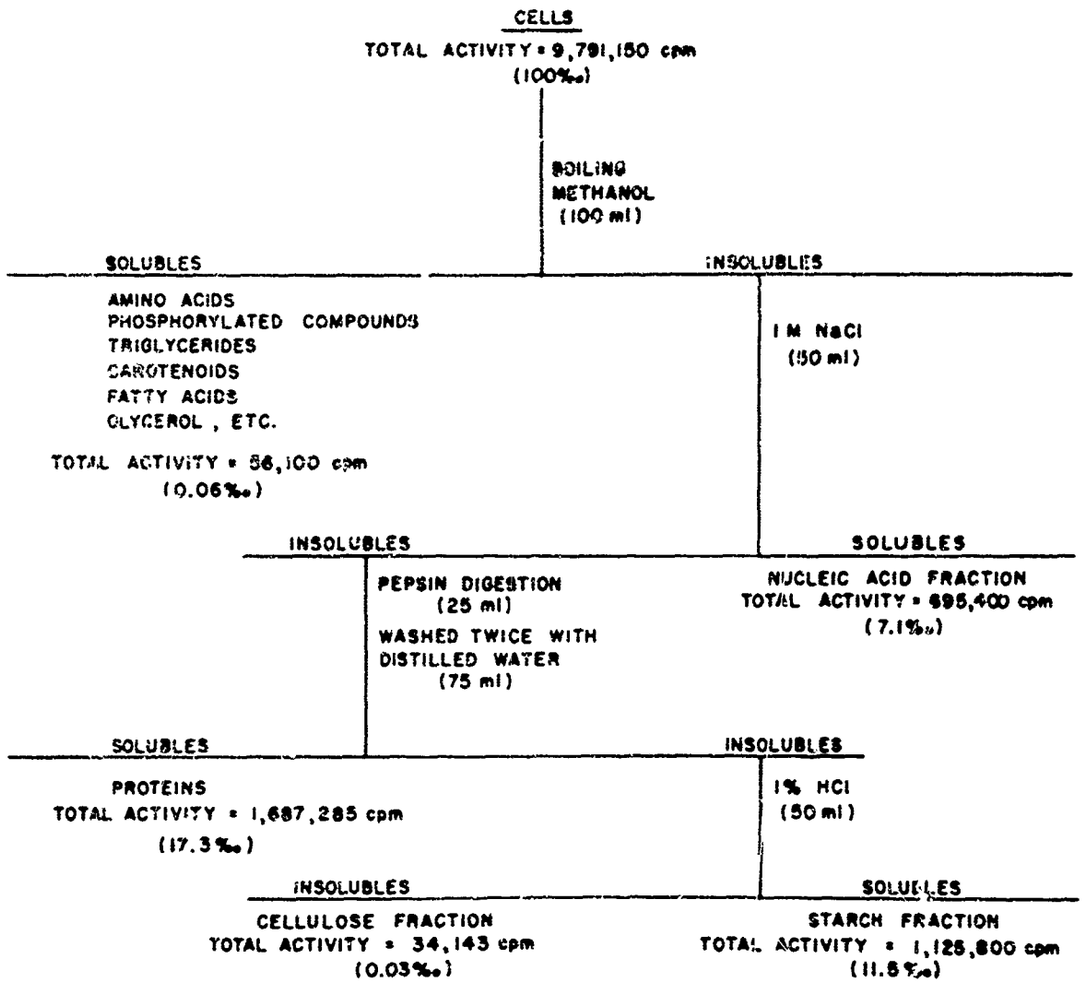


Fig. 7.10—Preferential extractions of Serratia marinorubra cells grown in radioactive medium.

Tabular results of this experiment are shown in Table 7.14, and a graphical summary is given in Fig. 7.10. As was found before (compare Tables 7.12 and 7.13), negligible quantities of activity were solubilized by boiling methanol. Approximately 7 per cent was found in the nucleic acid fraction, and 17 per cent was found in the soluble proteins. The activity liberated by hot 1 per cent HCl amounted to 11.5 per cent, and that liberated by the insoluble residues remaining was 0.03 per cent. These figures would indicate that most of the activity is to be found associated with nucleic acids, proteins, and starchlike compounds. Much activity, percentagewise, was not accounted for in the course of this procedure, and this, coupled with the fact that the extractions do not necessarily sharply define various fractions, would make these figures only generally indicative. Since the proteinaceous and starchlike materials seem to be primarily involved in retaining this activity, which is principally Y^{90}, it would seem reasonable to suspect chelation as an active force in Y^{90} uptake. The fact that a partition was effected shows very strongly that isotope uptake, or incorporation, does occur.

Figure 7.11 portrays graphically the decay characteristics of these cellular components. The original culture shows the characteristic Sr^{90}-Y^{90} mixture in equilibrium. The various partitioned components exhibit an excess of Y^{90} and some Sr^{90}. The final slurry, containing cellulose type compounds, decayed to a level below the sensitivity of the counting apparatus, indicating that nearly all, if not all, the activity of this residue was due to Y^{90}.

7.5 DISCUSSION

It is extremely difficult to extrapolate, especially quantitatively, from laboratory studies of biological systems to in situ conditions of the natural environment. The experiments reported here represent organisms growing at nearly optimum conditions in small culture vessels. Consequently, populations of a single species are realized, having numbers far exceeding those found in most natural environments. However, it is possible to ascertain the potential capabilities of such organisms in behaving in a given way under defined environmental conditions.

In biological isotope uptake there are certain environmental variables to be considered, such as temperature, pH, concentration of elements, and time. There is also radioactivity inherent in sea water, with such isotopes as K^{40}, Rb^{87}, C^{14}, etc., which could have effects in low-level counting. However, this source of radioactivity is not considered here. Radiation levels in these experiments are considered to be low enough to assume negligible radiation damage, and effects reported here are considered as the behavior of normal cells.

Autoradiography of bacterial colonies grown on radioactive media indicates qualitatively that concentration of the isotopes by the cells does occur. When colonies were grown on radioactive agar, this was very evident. The lack of clearly defined areas of concentration on MF's does not necessarily mean concentration did not occur. The small spots of concentration on the MF's could be related to capillary action of certain larger pores of the filter material or to compounds or complexes formed by these isotopes with constituents of the medium. These effects might conceivably have masked the effects produced by the colonies. Another important consideration is that the MF's were dried before autoradiography, whereas the colonies on agar had continual access to the moisture in the medium.

The basic concept in developing a technique for quantitation of uptake was to grow cells in radioactive medium and then measure the uptake. This measurement could have been done in either of two ways, measuring the loss of isotope from the medium or measuring the gain of activity by the cells. Evaluation of the loss of isotope from the medium, properly controlled, will indicate the loss from the medium due to the biological system considered, whereas measurement of the gain of activity of the cells indicates retention of isotopes by the cells. The two concepts are not necessarily identical since a biological entity can have indirect effects on the environment by altering pH and temperature and by utilizing some products and releasing still others. These changes would be difficult to differentiate by controls. By measuring the activity of the cells themselves, this error would be obviated.

Table 7.14—PREFERENTIAL EXTRACTIONS OF *Serratia marinorubra* CELLS GROWN IN RADIOACTIVE MEDIUM

	cpm/ml	Total ml	Total Activity		% Activity in Fraction 100% = 9,791,150 cpm	
Methanol slurry	*	50	*		-	
Methanol supernatant	561	100	56,100		0.06	
M NaCl slurry	195,721	50	9,786,050		99.94	
M NaCl supernatant	13,908	50	695,400		7.1	
Pepsin slurry	222,229	25	5,555,725		56.7	
Pepsin supernatant	32,357	25	808,925		8.3	
Distilled water wash 1	5,211	50	260,560	1,687,285	2.7	17.3
Distilled water wash 2	12,356	50	617,800		6.3	
1% HCl slurry	20,108	50	1,005,400		10.3	
1% HCl supernatant	22,516	50	1,125,800		11.5	
Distilled water slurry	1,707	20	34,143		0.03	

* When washed cells were added to boiling methanol, flocculation occurred, making representative sampling difficult. The combined activities of the M NaCl slurry and the boiling methanol fractions theoretically should equal this value, and are used here as the reference value for calculating percentages of total activity.

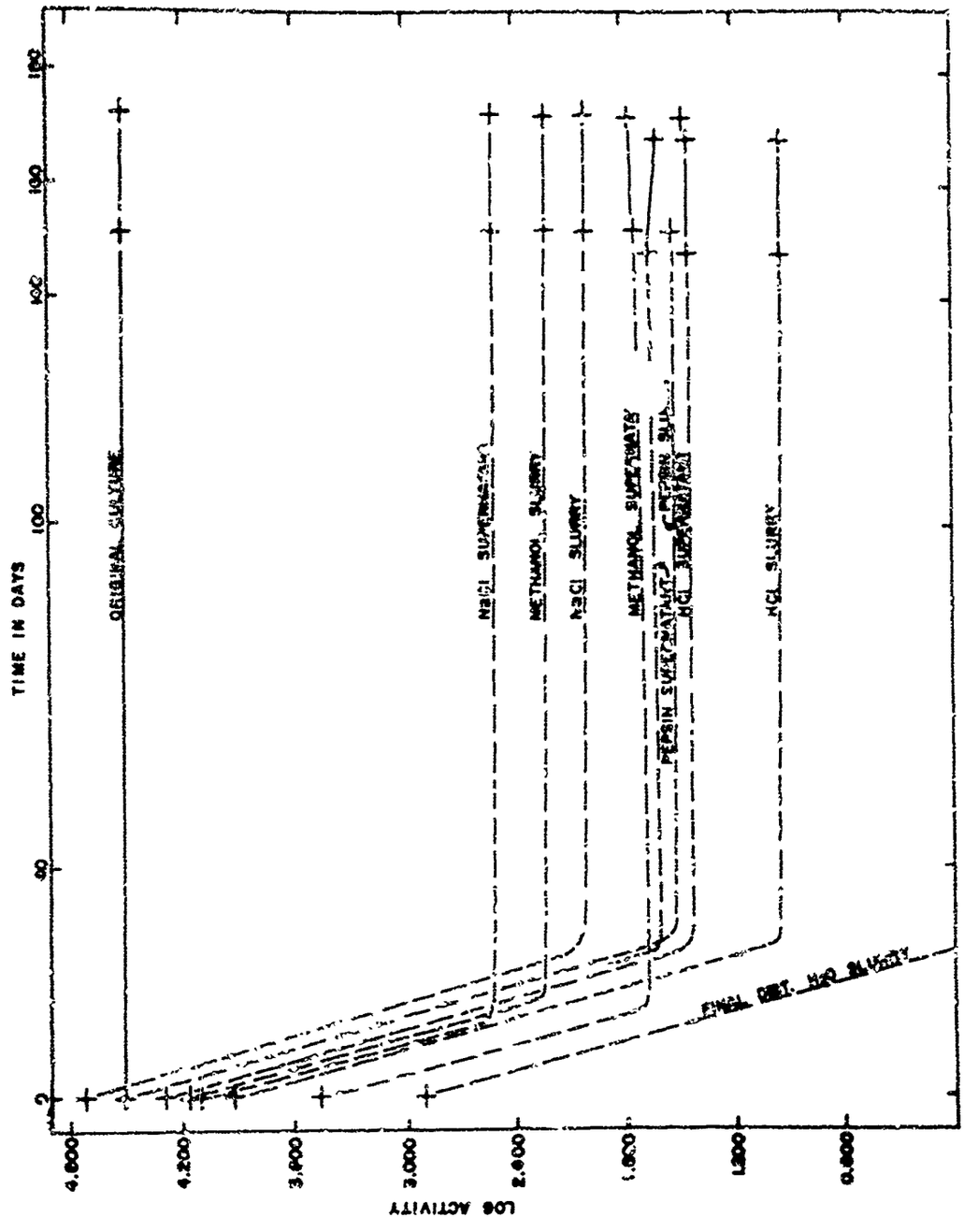


Fig. 7.11—Decay characteristics of chemical fractions of Serratia marinorubra cells.

Two procedures for separating cells from a medium were investigated: filtration and centrifugation. Centrifugation had advantages over filtration because of (1) less cost, (2) simpler counting geometry by virtue of using standard planchets throughout, and (3) the ability to sample both supernatant and centrifugate. The disadvantages of a centrifugation technique are (1) clear definition of phases is somewhat arbitrary in laboratory manipulation, (2) the cells tend to carry down sorbed isotopes, which would be difficult to correct by controls, and (3) dilution of the centrifugate is frequently necessary for counting, which introduces a dilution error.

The disadvantages of filtration are (1) the cost (currently 18.5 cents per filter), (2) the counting geometry is not standard throughout when filters and planchets are used, and (3) the fact that filtrates cannot be accurately evaluated due to loss of isotopes by sorption on the filtration apparatus. The advantages of filtration are (1) the definition of phases, i.e., cells and medium, are rather clearly defined, especially when washing is practiced, and (2) this procedure is more easily and accurately controlled. Generally filtration is more conservative of time than is centrifugation once a protocol has been established.

The decay of working solution would be negligible in the course of these experiments, less than 2 per cent at the end of the 11-month experimental period.

There is little doubt that uptake does occur. Every experiment, except autoradiography on MF's, indicated this. The percentage uptake, as shown in Table 7.1, ranged from 6 to 28 per cent of the original activity on the seventh day, which was the maximum for the 4.78×10^{-5} mc/ml (0.1-ml working solution) and 23.92×10^{-5} mc/ml (0.5-ml working solution) isotope levels. The maximum was apparently earlier, at four days, at the 47.84×10^{-5} mc/ml (1.0-ml working solution) level. The lower isotope levels generally retained greater percentages of activity. The other experiments cannot be accurately compared with this because they were designed primarily to test variables of the procedures; however, the percentage levels are generally comparable.

The amount of isotope sorbed on the cells was generally small, in the range of 2 to 3 per cent of the gross uptake, as indicated by the filtration experiments with various quantities of wash.

Population densities would affect the amount of retention, and the bacterial populations were approximately 5 to 6 million cells per milliliter, as estimated turbidimetrically. The population density of Platymonas subcordiformis was not determined.

In the interpretation of radioactivity counts, several factors must be considered. All values shown are corrected for background counts, and very low counting rates may be indistinguishable from background. In a study of background counts of 5, 10, and 20 min in duration, the range for the 5-min counts (41 determinations on separate days) was from 23.0 to 33.6 counts/min, with a mean of 28.6 counts/min. The range for the 10-min counts was from 25.9 to 32.2 counts/min, a mean of 28.8 counts/min. For the 20-min counts the range was 26.2 to 30.6 counts/min, with a mean of 28.4 counts/min. Most of the counting of experimental material was 10 min in duration, with background counts after every third or fourth sample. This would indicate that counts of 3 counts/min would have no practical validity and that counts of 10 counts/min would have doubtful validity.

Variations in efficiency of the counting apparatus were calculated from a standard calibrated UX_2 source. The apparatus is such that three counting positions can be used: position 1, nearest the window of the G-M tube; position 2, approximately 1 cm farther away from the G-M tube; and position 3, 1 cm farther than position 2. The calculated efficiency for position 1 was 31.87 per cent, and for position 2, 15.29 per cent. Counts made on 43 different days in position 3 show a mean efficiency of 7.47 per cent and a range of 6.80 to 7.97 per cent.

Coincidence loss was not corrected since the resolving time for this apparatus was 200 μsec, and coincidence loss at 30,000 counts/min would be approximately 9 per cent. Little of the experimental material approached this counting rate.

Counting geometry must be considered because the MF's were 47 mm in diameter and had a filtering diameter of 35 mm. The planchets used were approximately 31 mm in diameter. This could make counts of the MF's slightly low compared to planchets.

Very striking in these experiments was the selection of isotopes from the mixture, displayed by both Serratia marinorubra and Platymonas subcordiformis. Evaluation of the quantities of each isotope can be made from analyses of decay data. It should be pointed out that the dashed lines which form part of the decay curves in Figs. 7.7 to 7.9 and 7.11 indicate the presumed course of decay and that the solid lines are probable courses of decay. The dashed curves should be more rounded where the excess Y^{90} regains equilibrium with Sr^{90}. Computations are actually based on the counts at the time of sampling and the value of the equilibrium level, which is the level of no more appreciable decrease or increase in activity.

The ratio of activities of Sr^{90} and Y^{90} in equilibrium is 1 to 1, i.e., the short-half-life daughter product decays as rapidly as it is formed from the long-half-life parent. When there is an excess of Y^{90} above the equilibrium, a decrease of activity to a new equilibrium value, if any Sr^{90} remains, will occur. A deficiency of Y^{90} below equilibrium will show an increase of activity until equilibrium is attained.

The percentages of Y^{90} and Sr^{90} in Sec. 7.4 are percentages of activity. This is misleading as to the actual quantities of Sr^{90} and Y^{90} present. The decay relation for a long-half-life parent isotope and a short-half-life daughter isotope, Sr^{90} and Y^{90}, respectively, in this case, in equilibrium of decay, is shown by the formula:

$$\frac{N_{Sr^{90}}}{T_{Sr^{90}}} = \frac{N_{Y^{90}}}{T_{Y^{90}}}$$

where N is the number of atoms and T is the half life. Therefore, for one atom of Y^{90}, in equilibrium with Sr^{90},

$$N_{Sr^{90}} = \frac{(1)\ (9125)}{2.54} = 3592 \text{ atoms of } Sr^{90}$$

An estimation of the order of magnitude of the quantities of Sr^{90} and Y^{90} atoms can be made, based on several assumptions:

1. The energy of the calibrated source (95 per cent, 2.3 Mev; and 5 per cent, 1.5 Mev) is not appreciably different from the energy of the isotopes (Sr^{90}, 0.537 Mev; and Y^{90}, 2.18 Mev).
2. The counting efficiency in position 3 is 7.47 per cent.
3. The efficiency of counting of each isotope is in the same proportion in each position.
4. The final decay counts represent decay equilibria for Sr^{90} and Y^{90}. These assumptions are not necessarily true since Y^{90}, with greater energy, will be more efficiently counted than Sr^{90}. The G-M tube with a thin window, 1.9 mg/cm^2, will, however, approximate the order of magnitude of each isotope.

From the basic decay formula,

$$-\frac{dN}{dt} = \lambda N \qquad \text{and} \qquad \lambda = \frac{0.693}{T}$$

$$-\frac{dN}{dt} = \frac{0.693N}{T}$$

If $-(dN/dt)$ is the counting rate, uncorrected, for any one isotope (activity ratio for Sr^{90} and Y^{90} in equilibrium is 1 to 1, and if the half life, T, for Sr^{90} is 13,140,000 min, this makes the equation for the computation of the number of atoms of Sr^{90}

$$N_{Sr^{90}} = \frac{(\text{counts/min of } Sr^{90})\ (13{,}140{,}000)\ (\text{efficiency correction})}{0.693}$$

The counts per minute for Sr^{90} in equilibrium of decay are one-half the observed counting rate. The correction factor for an efficiency of 7.47 per cent would be 13.39 times the ob-

served counting rate. Knowing the counts per minute due to Sr^{90} in final equilibrium, the activity of Y^{90} will be the activity at the time of sampling (the first counting) less the counts per minute of Sr^{90} in final equilibrium of decay. The number of atoms of Y^{90} is calculated from the equation

$$N_{Y^{90}} = \frac{(\text{counts/min of } Y^{90})\ (3657.6)\ (\text{efficiency correction})}{0.693}$$

The numbers of atoms of Sr^{90} and Y^{90} were calculated from several representative experiments and are shown in Table 7.15. Referring to the data for Serratia marinorubra from Table 7.8 as recalculated in Table 7.15, it can be seen that 95.8 to 97.0 per cent of the activity retained was due to Y^{90} and that 3.0 to 4.7 per cent was due to Sr^{90}. When calculated on the basis of numbers of atoms, it can be seen that actually more atoms of Sr^{90} were retained than were atoms of Y^{90}, from 108 to 158 times as much Sr^{90}. It must be borne in mind, however, that 3592 times as many atoms of Sr^{90} were present, in equilibrium, in the medium initially.

The percentage-uptake values for atoms of Sr^{90} and Y^{90} show that greater percentages of atoms of Y^{90} than of Sr^{90} were retained. These same observations can be made for dialyzed Serratia marinorubra cells by comparing Tables 7.15 and 7.5.

Platymonas subcordiformis, on the other hand, retained greater numbers of atoms of Sr^{90} with respect to Y^{90} ($N_{Sr^{90}}/N_{Y^{90}}$), when compared to Serratia marinorubra. On an activity basis, 59.2 per cent was due to Y^{90}, and 40.8 per cent was due to Sr^{90}. Calculated on an atoms basis, Platymonas subcordiformis retained 3 to 4 per cent of the original Sr^{90} atoms and 5 to 6 per cent of the Y^{90} atoms.

Concentration factors were calculated on a basis of the activity in a given volume of cells compared with the activity in a comparable volume of medium, according to the equation

$$\text{Concentration factor} = \frac{\text{activity of volume of cells}}{\text{activity per ml of medium} \times \text{volume of cells}}$$

Where data were available, concentration factors for each isotope, i.e., Sr^{90} and Y^{90}, were calculated, as was the gross concentration factor. To make these calculations, several assumptions were necessary:

1. The population density of Serratia marinorubra cells was 5×10^8 bacteria/ml. This was established experimentally, by turbidimetry, in two cases but was estimated visually only in the remaining cases. A fluctuation from 3.5×10^8 to 7×10^8 cells/ml changes the concentration factor little, and in any case the same order of magnitude is shown.

2. The volume of a bacterial cell was assumed to be 4×10^{-12} cc, giving 20×10^{-4} ml of cells per milliliter of medium.

3. The efficiency of counting for both isotopes was assumed to be equal and equivalent to 7.47 per cent in position 3 and 31.87 per cent in position 1. This assumption introduces the most error into the calculations since, as was previously mentioned, the actual efficiencies for each isotope were not determined experimentally.

4. The final reading was assumed to be in equilibrium. Theoretically, decay equilibrium cannot be established since the rate of attaining equilibrium is asymptotic. However, the values for the final counts were not appreciably different.

5. In individual experiments some variables were not determined experimentally and were either assumed or calculated from theoretical considerations.

Concentration factors are shown in tabular form in Table 7.16. The data calculated from Tables 7.1 and 7.8, as shown in Table 7.16, are probably the most reliable of the data shown since the necessary variables were experimentally determined in these experiments. The data for the other experiments do compare favorably. It must be borne in mind that the order of magnitude only should be considered in this concept. As can be seen in Table 7.16, the trends for such variables as isotope level, pH, and washing of filters are the same as those discussed previously when computed on a percentage-activity basis.

Table 7.15 — CALCULATED NUMBERS OF ATOMS OF Sr^{90} AND Y^{90} RETAINED BY MICROORGANISMS

	Activity at Sampling cpm	Final Activity in Equilibrium cpm	cpm Sr^{90}	N Sr^{90} (X 10^{10})	% Total N Sr^{90}	cpm Y^{90}	N Y^{90} (X 10^{8})	% Total N Y^{90}	N Sr^{90} / N Y^{90}
S. marinorubra (data from Table 7.8)									
Total original activity	7027680	7027680	3513840	89181	100	3513840	2482.7	100	3594
Filter, pH 8.3	154776	13029	6514	165.38	0.18	148262	104.75	4.21	158
" pH 7.0	184800	12641	6320	160.41	0.17	178480	126.10	5.07	127
" pH 6.0	234360	13737	6868	174.32	0.19	227492	160.73	6.47	108
S. marinorubra, dialyzed (data from Table 7.5)									
Dialyzed cells in suspension	1361	314	157	0.934		1204	0.199		468
Dialysate	1256	1379	690	4.10		566	0.094		4381
Filter, no wash	12120	231	116	0.690		12004	1.984		35
" 50 ml wash	5412	63	32	0.190		5380	0.891		21
" 100 ml wash	10191	156	78	0.464		10113	1.675		28
Platymonas (data from Table 7.3)									
Total original activity	160880	160880	80440	2042	100	80440	56.83	100	3593
Initial culture	2011	2017	1008	25.58	1.25	1003	0.708	1.24	3614
Filter, no wash	8369	6588	3294	83.60	4.09	5075	3.58	6.30	2334
" 10 ml wash	7653	6111	3056	77.56	3.79	4597	3.24	5.70	2391
" 25 " "	7483	6092	3046	77.31	3.78	4437	3.13	5.50	2469
" 50 " "	7206	5921	2646	67.16	3.28	4560	3.22	5.66	2087
" 100 " "	6937	5898	2949	74.85	3.66	3988	2.81	4.95	2660

Table 7.16—CONCENTRATION FACTORS FOR *Serratia marinorubra*

Data from experiment shown in	Sample	Method	Isotope level mc/ml X 10^{-5}	Time in Days	Concentration Factors Gross	Sr^{90}	Y^{90}
Table 7.1	Washed filters	Filtration	4.78	2	3917		
	" "	"	"	4	13109		
	" "	"	"	7	10301		
	Filters not washed	"	"	2	4723		
	" "	"	"	7	12536		
	Washed filters	"	23.92	4	352		
	" "	"	"	7	9116		
	Filters not washed	"	"	4	9915		
	" " "	"	"	7	9758		
	Washed filters	"	47.84	4	7019		
	" "	"	"	7	2476		
	Filters not washed	"	"	4	7493		
	" " "	"	"	7	8948		
Table 7.3	Filter no wash	"	23.92	14	7440		
	" 25 ml wash	"	"	"	6255		
	50 " "	"	"	"	6333		
	" 75 " "	"	"	"	6357		
	" 100 " "	"	"	"	6005		
Table 7.5	Filter, no wash	"	23.92	19	44167		
	" 50 ml wash	"	(before washing cells)	"	23857		
	" 100 " "	"	"	"	40998		
Table 7.8	Filter, pH 8.3	"	59.8	ca. 14	15602	1313	29891
	" " 7.0	"	"	"	18628	1274	35892
	" " 6.0	"	"	"	23625	1385	45865
	Centrifugate, pH 8.3	Centrifugation	"	"	4501	366	8636
	" " 7.0	"	"	"	7439	1129	13749
	" " 6.0	"	"	"	7754	309	15199
	Centrifugate, pH 8.3 on filter	Filtration	"	"	6992	36	13947
	" " 7.0	"	"	"	12497	64	24930
	" " 6.0	"	"	"	12633	21	25244
Table 7.12		Centrifugation	5.98	5	1412		

The differences in orders of magnitude between filtration and centrifugation as means of separating cells from media are striking, generally differences by degrees from two to 10 times greater in filtration experiments.

The most probable gross concentration factors for *Serratia marinorubra* range from 6000 to 25,000, depending on the variables stated. The concentration factors for Y^{90} are much greater than for Sr^{90}.

Concentration factors for *Platymonas subcordiformis* were not calculated because population densities were not determined.

Concentration factors are identical when computed on the basis of relative activity and on the basis of numbers of atoms. Also, assuming a density of 1 for a bacterial cell, wet weight and volume are equivalent.

The reasons for the relatively great concentration of Y^{90} and the lesser concentration of Sr^{90} by *Serratia marinorubra* from sea-water media are difficult to postulate from evidence shown here. Strontium is a Group II element, with related elements such as Mg, Ca, and Ba. Yttrium is a Group III element, with Al, Sc, and La related. This greater concentration of Y^{90} might partially be explained by experimentally comparing the retention of its related elements by the organism.

Estimates of the abundance of Sr and Y in sea water[1] are 10 mg/liter for Sr and 0.0003 mg/liter for Y, a ratio of about 30,000 to 1. The isotopes as supplied in the medium were about 3600 to 1. This might conceivably have a bearing on this selective uptake.

A paradoxical situation arises in this uptake of Y^{90} by these organisms. Whereas the organisms selectively retained relatively great quantities of Y^{90}, these quantities of Y^{90}, in the course of a few relatively short half lives, become Zr^{90}, a Group IV element. This phenomenon of the effective change of elements could have practical applications in the study of cellular metabolism.

The mechanisms or forces involved in the retention of these isotopes by these unicellular organisms can be considered with respect to several properties.

Sorption on the cell surfaces occurs to some extent, as was shown by the relative activities retained after washing with varying quantities of wash. Percentagewise this would seem to be relatively small. When the filtration technique is used, there is some effective washing inherent in the filtration procedure itself, in which the first cells retained on the filter will have a flow of medium around the cells, with this effect becoming less as the aliquot continues to filter. If sorption were the primary factor, it would seem that maximum uptake would occur rapidly and not in the course of four to seven days as was found in one experiment.

A free ionic exchange of the isotope with the element within and outside the cell would indicate the normal levels of the particular element occurring in such cells under natural conditions. This probably would not be limited to the particular isotope. For example, Sr^{90} uptake in the presence of varied concentrations of Ca, Ba, and Mg, respectively, and in combination, might conceivably be affected. The same might apply to Y^{90} in the presence of Al, Sc, or La.

The extraction experiments, in which most of the activity was associated with constituents capable of chelation, indicate that this mechanism may be suspect. If the equilibrium constants for chelating compounds with Sr and Y are sufficiently great, it would seem plausible that such equations could be postulated:

$$Ch + Sr^{+2} \rightleftharpoons ChSr^{+2} + Sr^{90(+2)} \rightleftharpoons ChSr^{90(+2)} + Sr^{+2}$$

$$Ch + Y^{+3} \rightleftharpoons ChY^{+3} + Y^{90(+3)} \rightleftharpoons ChY^{90(+3)} + Y^{+3}$$

where Ch represents a compound capable of chelation; Sr^{+2}, nonradioactive Sr ions normally found in sea water; and Y^{+3}, normal nonradioactive Y ions. A free ionic exchange of the isotopes and nonradioactive ions within and outside the cell possibly explain, at least partially, the selective uptake of these ions. With relatively greater concentrations of Sr^{+2} in sea water, this chelation affinity could be partially if not completely fulfilled by Sr^{+2}, and $Sr^{90(+2)}$ would merely establish equilibrium.

On the other hand, Y^{+3} could be well below the level that would suffice its chelation affinities, and, when added as $Y^{90(+3)}$, it would result in the apparently great concentrations in the cells. The sea water used in all the experiments was from one lot, collected from the end of the Scripps Institution of Oceanography pier, La Jolla, Calif., filtered through a molecular filter, and stored in glass for use as needed. The Sr and Y concentrations in this sea water were not determined.

A related reaction, the ability of cell constituents to complex with these ions, should also be considered. It can be determined experimentally whether chelation or complexing, or both, can produce the concentration factors observed. Any definite statement as to the mechanism of uptake as observed would necessarily rest upon experimental determination of these phenomena.

Some literature is available relating to the uptake of Sr and Y by biological systems. Spooner[2] investigated the uptake by various marine algae and concluded that Sr is preferentially selected from a Sr^{90}-Y^{90} mixture by brown marine algae, with concentration factors from 14 to 40. The red and green algae selectively retained Y^{90} to the extent of depleting the available Y. A few preliminary experiments with unicellular algae indicate that Y^{90} is selected by Nitzschia, but no concentration factors were determined. Spooner's hypothesis is that

radioactive Sr is retained by ionic exchange and that radioactive Sr concentration reflects that relative amount of Sr already in the plants. His conclusion regarding Y uptake is that it is partly a matter of adsorption on surfaces and partly ionic exchange. His technique depended primarily on the loss of isotopes from the medium with some counts of biological material. The paper introduces an extensive discussion of radiobiological techniques and concepts.

Black and Mitchell[3] examined some Laminariaceae and Fucaceae spectrographically for trace elements and found concentration factors for Sr in these algae from 8 to 90.

Kaufman, Klein, and Greenberg[4] investigated the concentration of Sr^{89} in activated sludge and concluded that Sr^{89} uptake was complete in less than 30 min, with uptake reported as 50 to 55 per cent. This, of course, involved great quantities of inert material as well as biological entities.

The primary purpose of this work was to find suitable laboratory means for evaluating uptake or retention of isotopes, in vitro, by microorganisms. From the considerations given above, recommendations for establishing a protocol can be outlined:

1. The filtration method of separating cells from media is preferred over centrifugation both for reproducibility of conditions and for facility.
2. Complete radiological information should be evaluated, including:
   a. Resolving time of the apparatus.
   b. Background variations.
   c. Variations of efficiency, using each isotope under consideration.
   d. Absorption losses.
   e. Sample geometry should either be standardized or correlated.
3. Media showing a minimum of precipitation of isotopes should be used. Special attention in the cleaning of glassware should be observed.
4. Population densities should be routinely evaluated.
5. Environmental variables, such as pH and temperature, should be known or controlled.
6. Duplicate experimental conditions should be used, with duplicate sampling from each.
7. Time should be considered as a variable in each experiment.
8. Isotope levels should be such as to be easily and reliably counted, consistent with good health physics practices.
9. Decay curves should be accurately determined.
10. Evaluation of the biological entity rather than changes of the environment should be practiced.

7.6 SUMMARY

Several methods of evaluating the biological uptake of Sr^{90} and Y^{90} from sea water are described. The filtration method using molecular filters to separate bacteria from media proved to be the most reliable and practicable.

Autoradiographic techniques indicated that the bacteria concentrated the radioactive isotopes within the colony limits when cultivated on agar containing Sr^{90} and Y^{90}. The activity was removed if the colonies were washed off the plates before the emulsion was exposed.

Serratia marinorubra cells, when grown on radioactive media, were able to concentrate the activity from 6000 to 25,000 times. Of the activity retained, 95 per cent was due to Y^{90}, and 4 per cent was due to Sr^{90}. On a basis of atom uptake, more Sr^{90}, approximately 130 times as much, was found. The percentage values for concentration of atoms showed a greater concentration for Y^{90} than for Sr^{90}, presumably because of the 3592 to 1 ratio of Sr^{90} to Y^{90} in the medium initially.

Platymonas subcordiformis cultures selectively concentrated Y^{90} more than Sr^{90}. On an activity basis, 59.2 per cent was due to Y^{90}, and 40.8 per cent was due to Sr^{90}. Calculated on an atom-uptake basis, more Sr^{90} atoms were retained than Y^{90} atoms. On a percentage-atom-uptake basis, Y^{90} was concentrated to a greater degree.

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CHAPTER 8

CONSUMPTION OF MICROORGANISMS BY THE COPEPOD *Tigriopus californicus*

By Donald W. Lear, Jr., and Carl H. Openheimer, Jr.

Microorganisms labeled with Sr^{90} and Y^{90} and placed in the presence of higher organisms of the food chain can potentially indicate several things: (1) whether such microorganisms can be consumed by the higher organisms, (2) whether the higher organisms themselves become radioactive by the ingestion or digestion of the tagged microorganisms, and (3) the nutritional significance of the microorganisms to the higher organisms. Experiments were devised to investigate the first two of these problems.

The harpacticoid copepod *Tigriopus californicus* grows only, as far as is known, in splash pools, i.e., pools above the high-tide mark and dependent upon splash for sea-water replenishment. This environment is characterized by relatively extreme fluctuations of temperature and salinity, with occasional desiccation. The *Tigriopus californicus* copepods used were collected from splash pools on a shelf rock, a large shale formation along the foot of the cliffs approximately 100 yards north of the Scripps Institution of Oceanography pier in La Jolla, Calif.

The copepods were washed by placing them in one section of a Spray anaerobe dish; the opposing section of the dish was darkened by placing black electrical tape on the outside walls and bottom (Fig. 8.1). Sterile, molecular-filtered sea water was placed in the darkened section as wash. When placed with the light side of the dish near a window, the negative phototropism of the copepods caused them to migrate over the transverse ridge of the Spray dish to the clean water on the darkened side. This procedure was repeated several times to thoroughly wash the copepods, which were then placed in clean petri dishes containing sterile, molecular-filtered sea water and were left for 24 hr to starve before initiating the experiments.

In the first experiment, washed cells of the marine bacterium *Serratia marinorubra*, grown in radioactive Sr^{90}-Y^{90} medium, were added to a culture of 50 *Tigriopus californicus* copepods in 100 ml of sterile molecular-filtered sea water. This was done in duplicate, with a control dish containing bacteria but no *Tigriopus* organisms. The activity of the cultures initially was counted on planchets in a Nuclear-Chicago model 182AX scaler with a G-M tube. Standard bacteriological counts in agar pour plates were made, using sea water–peptone agar. After 24 hr, bacteriological counts and radiological counts were again made. In addition, the *Tigriopus* organisms were removed with a medicine dropper, washed in 50 ml of sterile molecular-filtered sea water, placed on planchets, and counted.

The results of this experiment are shown in Table 8.1. It should be noted that all copepods in duplicate culture II were dead at the end of the 24-hr period and that all in culture I were

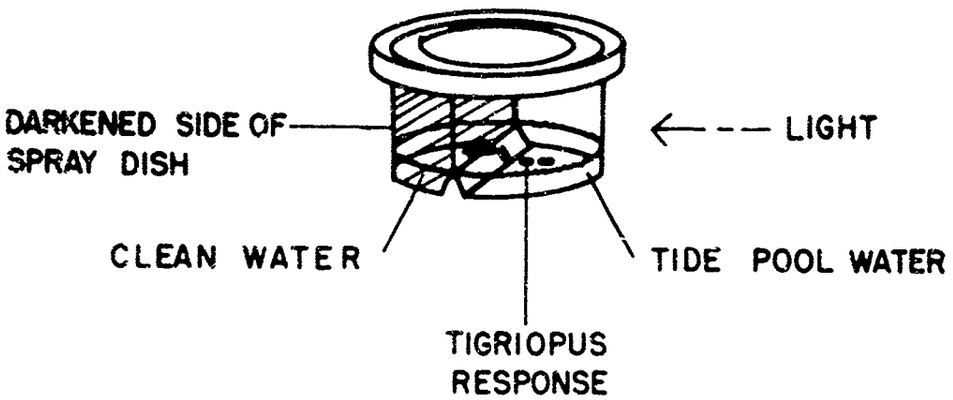

Fig. 8.1—Apparatus for washing copepods.

Table 8.1—CONSUMPTION OF Serratia marinorubra BY Tigriopus californicus

	Culture I		Culture II		Control		Tigriopus
	bact./ml	cpm/ml	bact./ml	cpm/ml	bact./ml	cpm/ml	cpm/ml
Initially	2,900,000	7	2,255,000	16	3,055,000	8	-
24 hours	2,605,000 (Tigriopus alive)	9	4,490,000 (Tigriopus dead)	9	1,165,000	7	0

alive and active. The appearance of the bacterial colonies was noted during counting, and their appearance was uniformly alike, indicating that the level of extraneous bacterial contamination was low.

Some indications are shown from the bacteriological counts, but the data are too scarce, with too many variables, to draw any valid conclusions. The bacterial population in the control dish dropped to approximately one-half its original value, whereas these populations remained the same in the culture with the Tigriopus organisms alive and doubled with the Tigriopus organisms dead. In the case of the increase with the Tigriopus organisms dead, the decay of the dead organisms would be accountable. Three possibilities exist for the culture with living Tigriopus organisms, in which the bacterial populations remained the same:

1. The population could have increased and been grazed down to the level found.
2. The population could have grown, nurtured with organic exudates, excrement, etc., from the Tigriopus organisms and not been grazed.
3. The population could have stabilized with the nutrient supplied by the Tigriopus organisms and not been grazed.

Although no firm conclusion can be drawn, with due consideration to all facts, it seems plausible that Tigriopus californicus organisms do not consume the Serratia marinorubra cells to any appreciable extent.

The radiological counts in this case are too low to be of any consideration. This in itself may be indicative that the Tigriopus organisms did not concentrate the isotopes to any degree, either through ingestion and assimilation of the bacteria or through simple ion-exchange mechanisms.

In a second experiment, done in the same manner, a bacteria-free culture of the green alga Platymonas subcordiformis was grown in sea water containing Beijerinck's medium with Sr^{90} and Y^{90} added. The cells were harvested, washed, and added to 50 sterile molecular-filtered sea-water cultures containing 100 washed Tigriopus organisms. Duplicate cultures and duplicate controls, containing the algae but no Tigriopus organisms, were used. Direct microscopic counts of Platymonas subcordiformis cells were made in a Petroff-Hauser chamber, and radioactivity counts were made on planchets. The duration of this experiment was 48 hr, with subsequent radioactivity counting to follow decay curves. Table 8.2 shows the results of this experiment.

Table 8.2—CONSUMPTION OF Platymonas subcordiformis BY Tigriopus californicus

	Initially		48 Hours		Washed Tigriopus
	Cells/ml	Cpm/ml	Cells/ml	Cpm/ml	Cpm
Culture I	10,790	210	1,550	316	305
" II	9,130	184	2,050	300	284
Control I	*	172	50,000	554	-
" II	*	108	50,000	317	-

*Not counted; should be same as other initial inocula.

From the direct microscopic estimations of the Platymonas populations, it is quite clear that these microorganisms were actively grazed by the Tigriopus organisms. From an initial population of approximately 10,000 Platymonas cells per milliliter, the cultures containing Tigriopus organisms dropped to approximately 2000 per milliliter, a decrease of 80 per cent. Control populations, on the other hand, are at least five times greater than initially.

The radiological counts in this case do not give as clear a picture of this situation. The initial activity level of 100 to 200 counts/min/ml apparently increased to 300 to 350 counts/

min/ml. This increase of activity is to a great extent due to a deficiency of Y^{90} below decay equilibrium, as introduced in the cells. A deficiency of Y^{90} below equilibrium will increase in activity until equilibrium is established with the long-lived parent isotope Sr^{90}, when the activity ratio will be 1 to 1 for each isotope; then the characteristic decay of the parent Sr^{90}, of 25-year half life, will be shown. This difference in activity is also due to sampling, i.e., by insufficiently mixing the cells in the culture before sampling. It should be noted, however, that duplicate samplings were made in all cases, with close agreement between replicates.

From the radiological counts it can be seen that appreciable activity was gained in 48 hr by the Tigriopus organisms. The counts for these organisms are probably low owing to absorption of beta particles in the relatively dense matter of the organisms.

By analyzing decay curves it can be shown that the Platymonas cells as introduced into the cultures were deficient in Y^{90} below decay equilibrium, i.e., the Platymonas cells concentrated more Sr^{90} than Y^{90} from the culture medium. Calculations are shown in Table 8.3. For Sr^{90} and Y^{90} in decay equilibrium, the activity ratio is 50-50. As can be seen, initially it was

Table 8.3—CALCULATION OF AMOUNTS OF Sr^{90} AND Y^{90} ISOTOPES FROM DECAY DATA

	Activity		Number of Atoms		
	% Sr^{90}	% Y^{90}	Sr^{90} (X 10^6)	Y^{90} (X 10^6)	$\frac{Sr^{90}}{Y^{90}}$
Culture I, Initially	27.2	72.8	6791.9	5.05	1343
" II, "	27.2	72.8	5949.0	4.44	1340
Control I, "	21.5	78.5	4402.3	4.47	985
" II, "	18.5	81.5	2379.6	2.91	816
Culture I, 48 hours	43.4	56.6	16300.3	5.91	2757
" II, "	40.3	59.7	14396.6	5.93	2428
Control I, "	43.4	56.6	28614.7	10.38	2756
" II, "	37.1	62.9	13980.2	6.59	2121
Tigriopus I, "	16.1	83.9	2915.0	4.24	688
" II, "	9.2	90.8	1546.7	4.27	362

approximately 25-75, and after 48 hr, 40-60, indicating a partial restoration to decay equilibrium. The Tigriopus organisms, on the other hand, apparently concentrated the Y^{90} isotope more than the Sr^{90}. However, the beta energy of Sr^{90} is 0.537 Mev, and that of Y^{90} is 2.18 Mev. This may exaggerate the decay picture for the Tigriopus organisms owing to absorption of the weaker Sr^{90} beta particles in the relatively dense matter of the Tigriopus organisms.

The relative numbers of atoms of Sr^{90} and Y^{90} in each sample were calculated, primarily to show that the relative activities of these two isotopes are not necessarily indicative of the chemical processes that took place. They show the same patterns as percentage activities, but more accurately they represent relative proportions of the isotopes.

Calculations of the grazing of Platymonas cells by Tigriopus organisms can be made on the basis of direct microscopic counts and of radiological counts. In each culture, approximately 10,000 Platymonas cells per milliliter were present initially, with a total population in each culture of approximately 500,000 cells. After 48 hr the Platymonas populations in cultures with Tigriopus organisms dropped to approximately 100,000, whereas the populations

in control cultures with no Tigriopus organisms rose to over 2,500,000 cells. Assuming this latter figure to be representative, the difference in populations would be the quantity grazed, or 2,400,000 cells in 48 hr. Since there were very nearly 100 Tigriopus organisms in each culture by actual count, this would average to 24,000 cells eaten by each Tigriopus organism in 48 hr.

The calculations based on measurements of radioactivity are complicated by the apparent increase of activity in the cultures, the unknown quantities of isotopes capable of diffusion from the cells into the culture medium, and the absorption in Tigriopus organisms of beta particles. Assuming an average value for the activity initially of 300 counts/min per milliliter of culture, or a total activity of 15,000 counts/min in each culture, and also assuming that this activity is carried solely by the Platymonas cells, with 500,000 cells initially, leads to a calculated activity of 0.03 count/min/cell. The activity of 100 washed Tigriopus organisms at the end of the experiment was approximately 300 counts/min. This activity represents, therefore, 300/0.03, or 10,000 cells ingested in 48 hr. As was noted, the measured activity of the Tigriopus organisms is probably low, which means that a greater number of cells was probably ingested.

If this is calculated on a basis of 200 counts/min per milliliter of culture initially, or a total of 10,000 counts/min, the result is 15,000 cells grazed by the Tigriopus population in 48 hr.

As can be seen, the direct counts yield an average figure of 24,000 cells per Tigriopus organism in 48 hr, and radiological counts yield 1000 to 1500 cells per Tigriopus organism, a difference of a factor of approximately 20. The direct counts are probably more reliable because of the numerous difficulties with which the radiological counts were beset.

Marshall and Orr[1] fed a number of unicellular algae to Calanus, using P^{32}-labeled microorganisms. Among their conclusions it was noted that Calanus was capable of ingesting up to 50,000 Platymonas cells per day, which is in good agreement with the order of magnitude found here. They also concluded that organisms below 10 μ could not be readily ingested, which would substantiate the tentative conclusions concerning bacteria found here.

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CHAPTER 9

UPTAKE OF FISSION PRODUCTS BY PHYTOPLANKTON

By W. H. Thomas, Donald W. Lear, Jr., and F. T. Haxo

9.1 INTRODUCTION

The initial plans for this project included (1) the isolation of single phytoplankton cells after the test explosion and the measurement of their radioactivity and (2) the measurement of the activity taken up from active sea water added to unialgal cultures of a diatom and a dinoflagellate which were maintained aboard ship. Both these approaches were discarded after technical difficulties in isolating phytoplankton at sea were experienced and also because the diatom and dinoflagellate cultures failed to survive at sea. Instead, radioactive sea water was transported back to the Scripps Institution of Oceanography laboratories by one of us (F. T. Haxo) and was used in the experiments described below.

These experiments were conducted at the Scripps Institution of Oceanography during the period May 20 to 30, 1955, to determine the amount of radioactivity taken up by laboratory cultures of a marine dinoflagellate from radioactive sea water collected in the test area.

9.2 MATERIALS

Cells of Gonyaulax polyedra were grown in unialgal culture at 25°C and approximately 500 foot-candles of light in the following nutrient medium:

75 per cent aged sea water	2×10^{-4}M K_2HPO_4
2 per cent soil extract	10^{-6}M $MnCl_2$
10^{-3} per cent EDTA-Na_2	10^{-5}M $FeCl_3$
2×10^{-3}M KNO_3	

After approximately 10 days of growth the cells were concentrated by filtration on sintered glass. Such cells had just finished their logarithmic growth phase. The cells were removed from the concentrate by light centrifugation and resuspended in 75 per cent aged and filtered nonradioactive sea water which was collected in the pelagic area one month before the test (collected Apr. 15, 1955, by Robert Holmes) and which contained 2×10^{-6}M K_2HPO_4 and 2×10^{-3}M KNO_3. The cells were then washed three times by centrifugation and resuspension. After this initial treatment the cells had lost their motility, but they regained it during the experiment.

The radioactive sea water used in the experiment was obtained from a depth of 125 meters at station 4 on May 15, 1955. It had a specific activity of 70,000 counts/min/ml and was designated ripple 1.

9.3 TREATMENTS

Gonyaulax cells were cultured in a 100-ml final volume of the above-mentioned suspending medium containing 50, 25, and 5 per cent radioactive sea water. The cultures were inoculated with 10 ml of cell suspension so that the initial cell concentration in each culture was approximately 675 cells/ml. Controls consisted of cultures without radioactive sea water and of flasks of radioactive medium without cells. The latter were of particular value in assessing the amount of activity incorporated into precipitate formed during the experiment.

After inoculation the cultures were incubated at 20°C and approximately 500 foot-candles of continuous light.

9.4 SAMPLING AND COUNTING

Two 0.1-ml aliquots of the original radioactive sea water were placed on a planchet and dried. These were counted on the shelf closest to the window of the G-M tube and were recounted at various times throughout the experiment to obtain decay curves. A 0.5-ml portion of the initial medium was plated and counted after inoculation in order to obtain the initial activity in each flask. At each sampling time the control treatments without cells were similarly plated on planchets and counted. These counts showed that no activity was adsorbed on the glass walls of the flasks. Samples for determining uptake were taken at 9, 21, 43, and 91 hr. A 5-ml portion of the culture was filtered through type HA millipore filters. The filter was washed five times with 5 ml of 75 per cent nonradioactive sea water and then was dried and counted directly using a large planchet holder. Similar treatment of radioactive controls without cells allowed the amount of radioactivity in any precipitate which was formed to be determined. Assuming that the cells had no effect on the amount of precipitate formed, one could then calculate the amount taken up by the cells.

Counting of radioactivity was done using a Nuclear-Chicago 182AX scaler with a regulated voltage input and a Tracerlab TGC2 G-M tube having a 1.9 mg/cm^2 mica end window. Such a counting system is 98 per cent efficient for β radiations which enter the tube but only 1 per cent efficient for γ radiation. Cell counts were made at 21, 43, and 91 hr.

9.5 RESULTS AND DISCUSSION

9.5.1 Condition of Cells

Gonyaulax polyedra requires for growth an exogenous supply of one or more organic growth factors. This requirement is met in laboratory culture by a supplement of soil extract. Such factors accumulated in excess by the cells during previous growth could presumably have been eliminated during the washing procedure. Since the suspending medium used in the uptake experiments contained no soil extract, it was expected that further growth would be limited. Cell counts support this contention. The average number of cells per milliliter at 20 hr after inoculation was 349; at 40 hr, 352; and at 90 hr, 294. The average of all cell counts, 333 per milliliter, was used in calculating concentration factors. Although not growing, the cultures were still alive since the cells retained motility throughout the experiment.

9.5.2 Precipitate Interference

Since it was expected that a certain amount of activity would be occluded on any precipitate which was formed and would be counted on the filter along with the activity in the cells, radiochemical controls containing no cells were included. In calculating the true amount of activity in the cells, the activity in this precipitate at each sampling time was subtracted from that in the cells. As mentioned previously, it was assumed that the cells would have no effect on precipitate formation. That this is not a valid assumption was shown by the 90-hr sampling. About 25 to 35 per cent more activity was found in the precipitate than in the cells plus pre-

cipitate at this time. Thus the phytoplankton prevented the formation of this precipitate. This could be due to the lowering of phosphate concentration in the medium owing to phosphate uptake by the cells. It is not possible by using these data to evaluate the effect of the cells on the precipitate, but the net effect would be to increase the calculated value of the activity in the cells.

However, this error in the calculated activity in the cells during the first 40 hr may be small since the amount of activity precipitated in the radiochemical control flasks was low during the initial 40 hr as compared with the 90-hr values. The precipitate data are shown in Table 9.1, and the data on uptake by the cells are given in Table 9.2.

Table 9.1 — ACTIVITY RETAINED IN PRECIPITATES AFTER FILTRATION OF 5 ML ON HA MILLIPORE FILTERS

Initial activity cpm/5ml	Activity (corrected for decay) retained by filtration of 5 ml on HA millipore filters (pore size approximately 0.5 μ) at			
	9 Hours Cpm/5ml	21 Hours Cpm/5ml	40 Hours Cpm/5ml	90 Hours Cpm/5ml
45325	739 (1.63%)	1398 (3.08%)	1730 (3.82%)	8986 (19.82%)
22095	325 (1.47%)	507 (2.29%)	1174 (5.31%)	4879 (22.08%)
4710	65 (1.38%)	91 (1.93%)	123 (2.61%)	944 (20.04%)

Uptake by cells: The results of this experiment are shown in Table 9.2.

At the end of the experiment (145 hr) an attempt was made to evaluate the precipitate error by washing the filter pads with sea water adjusted to pH 7.4, 6.0, 3.7, and 2.5. The results cannot be evaluated without more data, but in general the most activity was removed from pads having the lowest initial activity, from pads having no cells, and from pads for which the lowest pH washes were used. Since 40 to 60 per cent of the activity was removed at pH 2.5 from pads having both cells and precipitate, it is estimated that about 50 per cent of the activity is adsorbed on the cell surface and that 50 per cent is incorporated into the cell.

An attempt was made using Edward D. Goldberg's pulse-height analyzer to identify the active isotopes taken up from this mixture of fission products. Although it was not possible to identify these isotopes, certain peaks appeared in the samples (see Figs. 9.1 to 9.6). Four main peaks were apparent, at channels 25, 40, 65, and 90. The data shown in Figs. 9.3 to 9.5 are of particular interest. Figure 9.3 shows data from the cells and the precipitate, Fig. 9.4 shows those from the precipitate only, and Fig. 9.5 is the difference between Figs. 9.3 and 9.4. The peaks at channels 24 and 90 are due to the precipitate, and those at 40 (34) and 60 (52) are due to the cells. Such data are only suggestive of differential uptake since a peak may be due to radiation from more than one isotope and since its position may shift owing to the vagaries of the electronic gear used.

Caution should be exercised in extending these data to the effects of a test explosion on fission-product uptake by phytoplankton. First, this was not a growing culture. A growing population would be expected to be metabolically more active and thus might take up more radioactivity. Second, the organism used normally grows in areas near the shore, not in a pelagic zone. It is also not a diatom, a group which would be expected to make up the bulk of the phytoplankton of the test area. Data bearing on these points were obtained by Leo Berner.

(Text continues on page 110.)

Table 3.2—ACTIVITY TAKEN UP BY CELLS

	Time After Inoculation 9 Hours	21 Hours	40 Hours
50% Radioactive Sea Water			
Initial Activity * 40310 cpm/5ml			
Activity retained * by filtration of 5 ml (cpm)	1406	2301	3846
% taken up	5.15%	5.71%	9.54%
Concentration factor ** cpm/ml cells ÷ cpm/ml water	2075	3396	5677
Uptake per cell cpm/cell	0.83	1.36	2.27
25% Radioactive Sea Water			
Initial Activity = 20960 cpm/5ml			
Activity retained * by filtration of 5 ml (cpm)	649	1734	1804
% taken up	3.10%	3.27%	6.09%
Concentration factor ** cpm/ml cells ÷ cpm/ml water	1843	4923	5122
Uptake per cell cpm/cell	0.24	1.02	1.07
5% Radioactive Sea Water			
Initial Activity = 4385 cpm/5ml			
Activity retained * by filtration of 5 ml (cpm)	151	341	503
% taken up	3.44%	7.77%	11.47%
Concentration factor ** cpm/ml cells ÷ cpm/ml water	2049	4627	6825
Uptake per cell cpm/cell	0.089	0.20	0.30

* Activities corrected for precipitate formation and for decay.
** The relation: 10^6 cells = 0.0497 ml was used in this calculation.

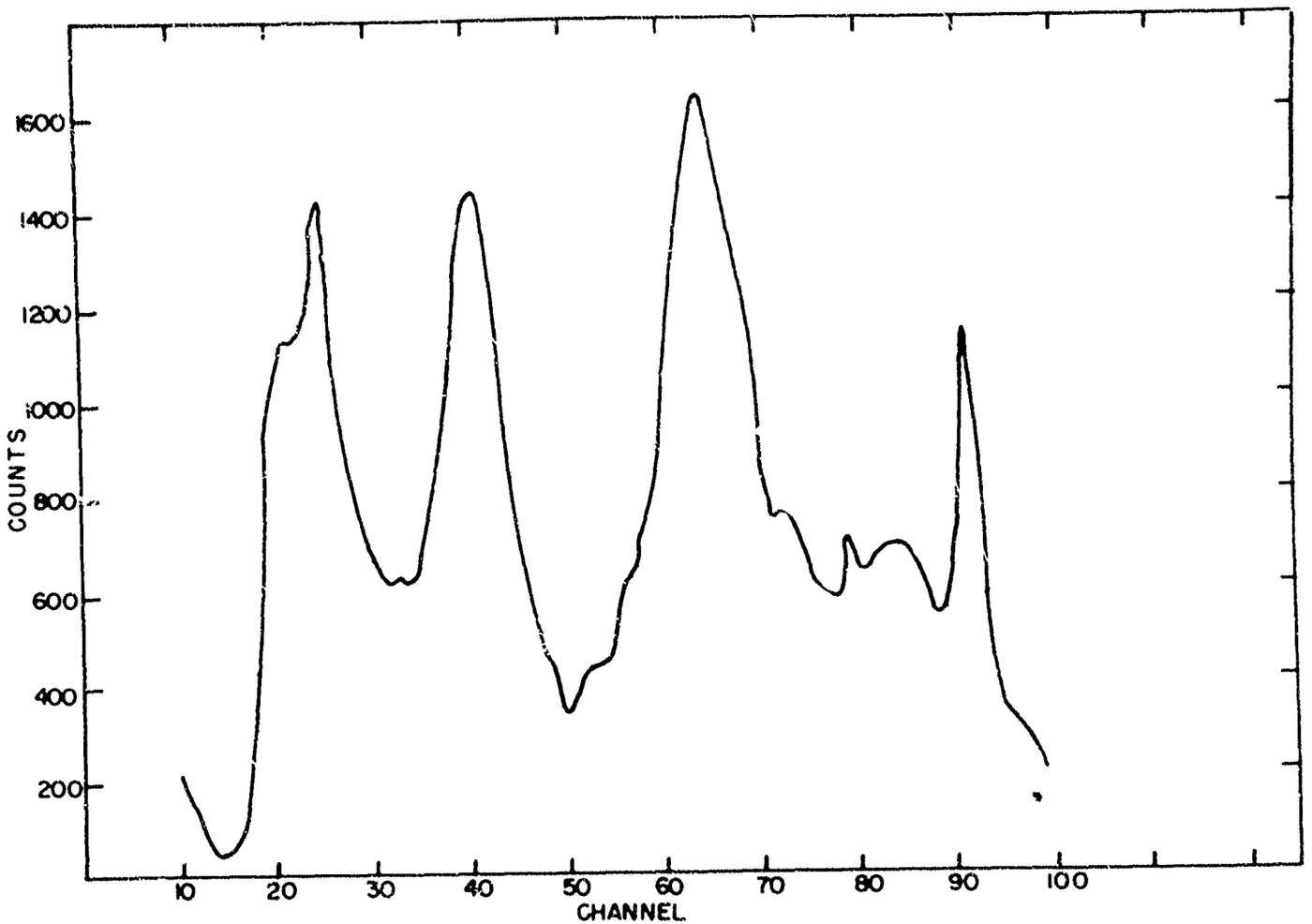

Fig. 9.1—Energy spectrum of total harvest from most active samples at 145 hr after inoculation into 50 per cent radioactive sea water. Cells plus precipitate; harvest A; 1.5-min run at 1630, May 31, 1955.

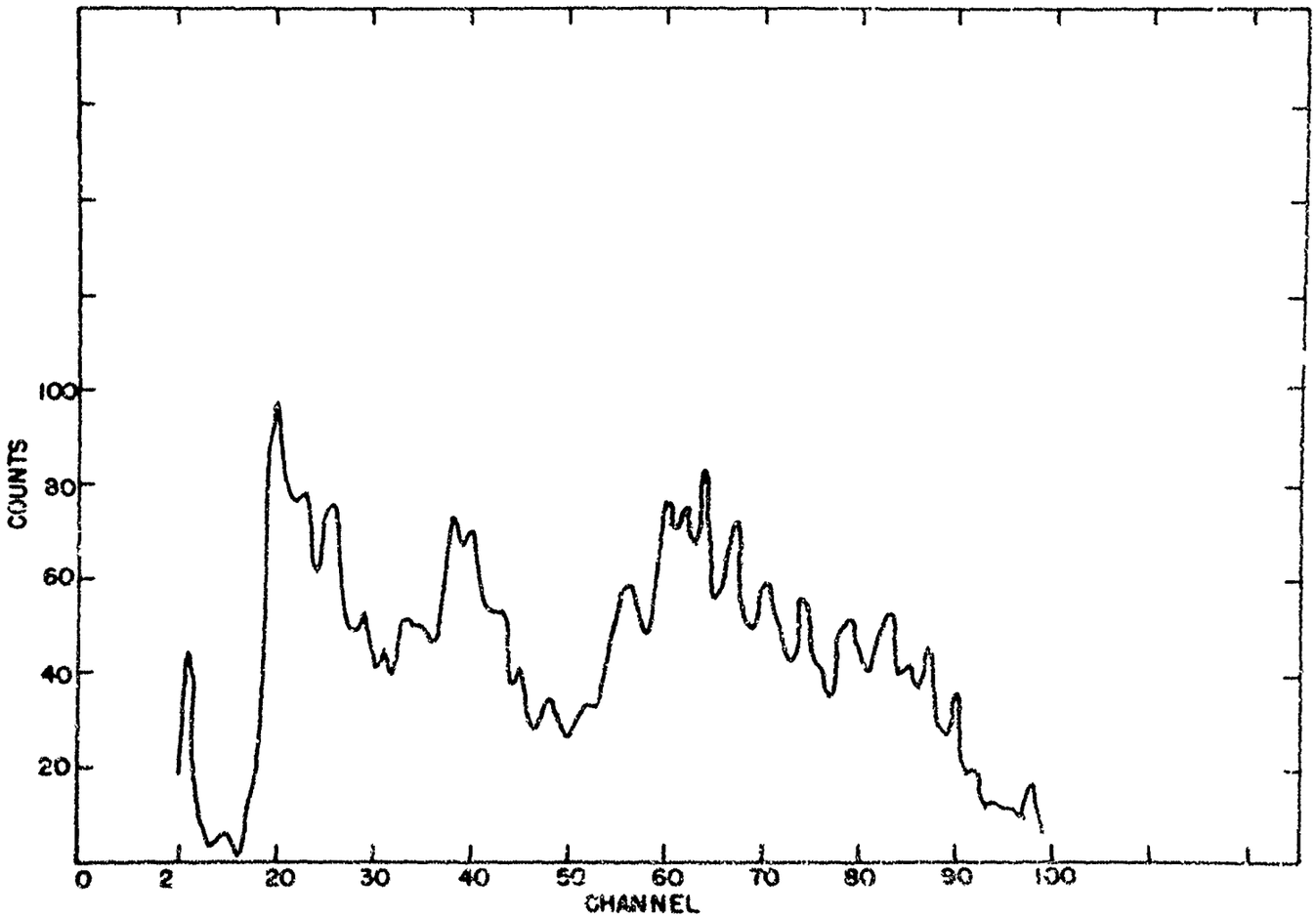

Fig. 9.2—Energy spectrum of sample taken 40 hr after inoculation. Cells plus precipitate; 3-min run at 1600, June 2, 1955.

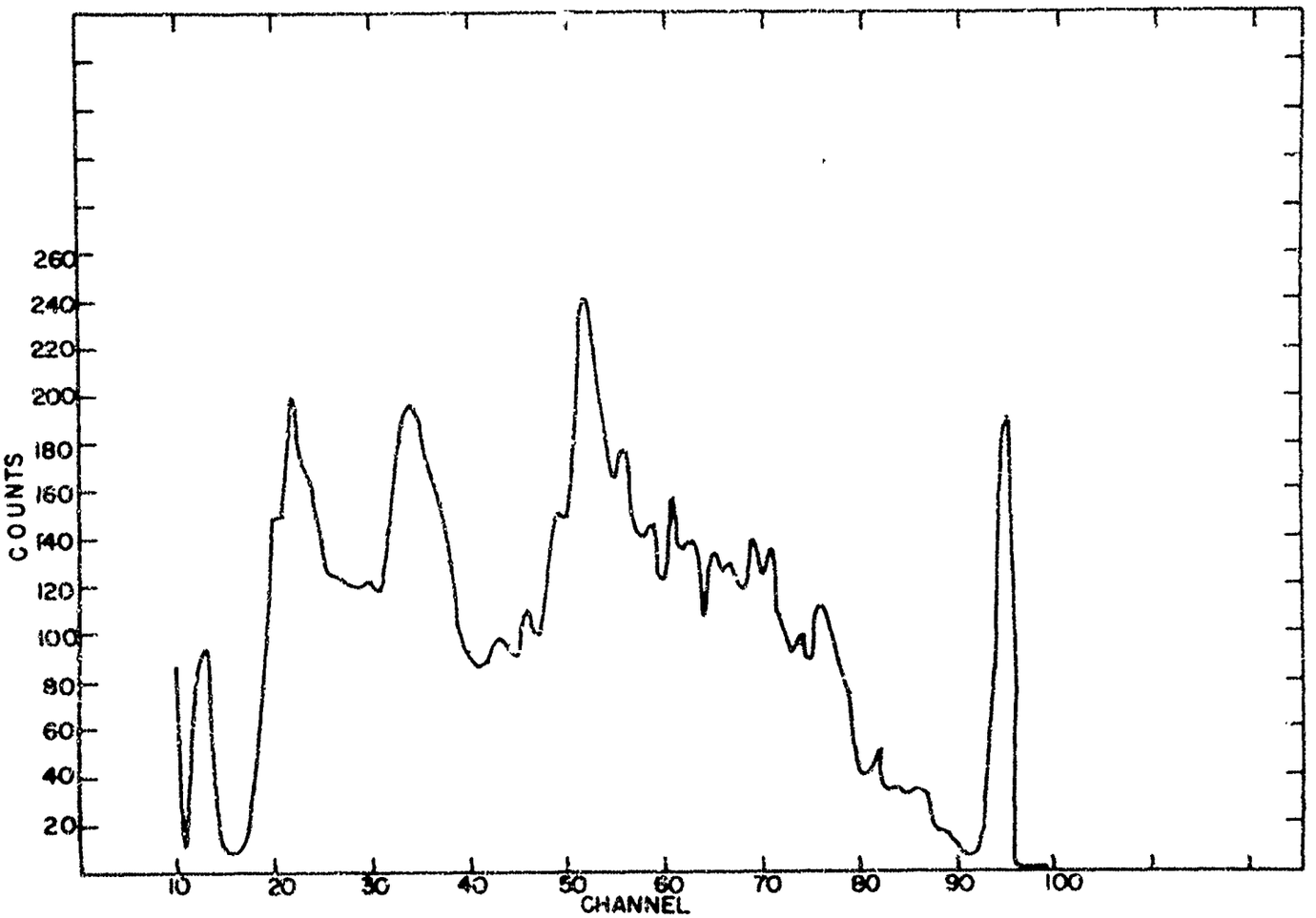

Fig. 9.3—Energy spectrum from sample taken 40 hr after inoculation into 50 per cent radioactive sea water. Cells plus precipitate; 9-min run, June 13, 1955.

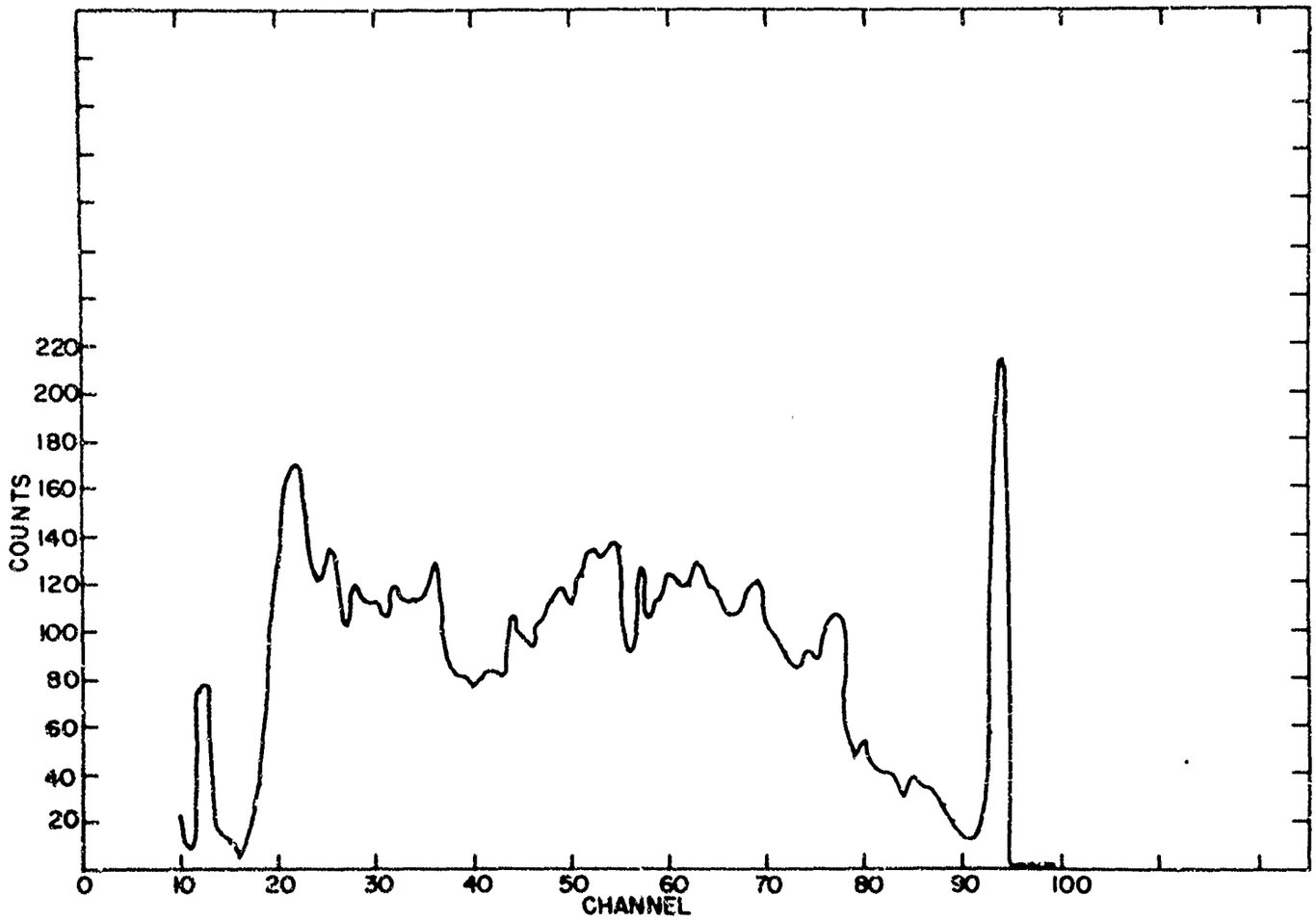

Fig. 9.4—Energy spectrum of precipitate formed 40 hr after experiment started. Fifty per cent radioactive sea water; precipitate only; 9-min run at 2100, June 14, 1955.

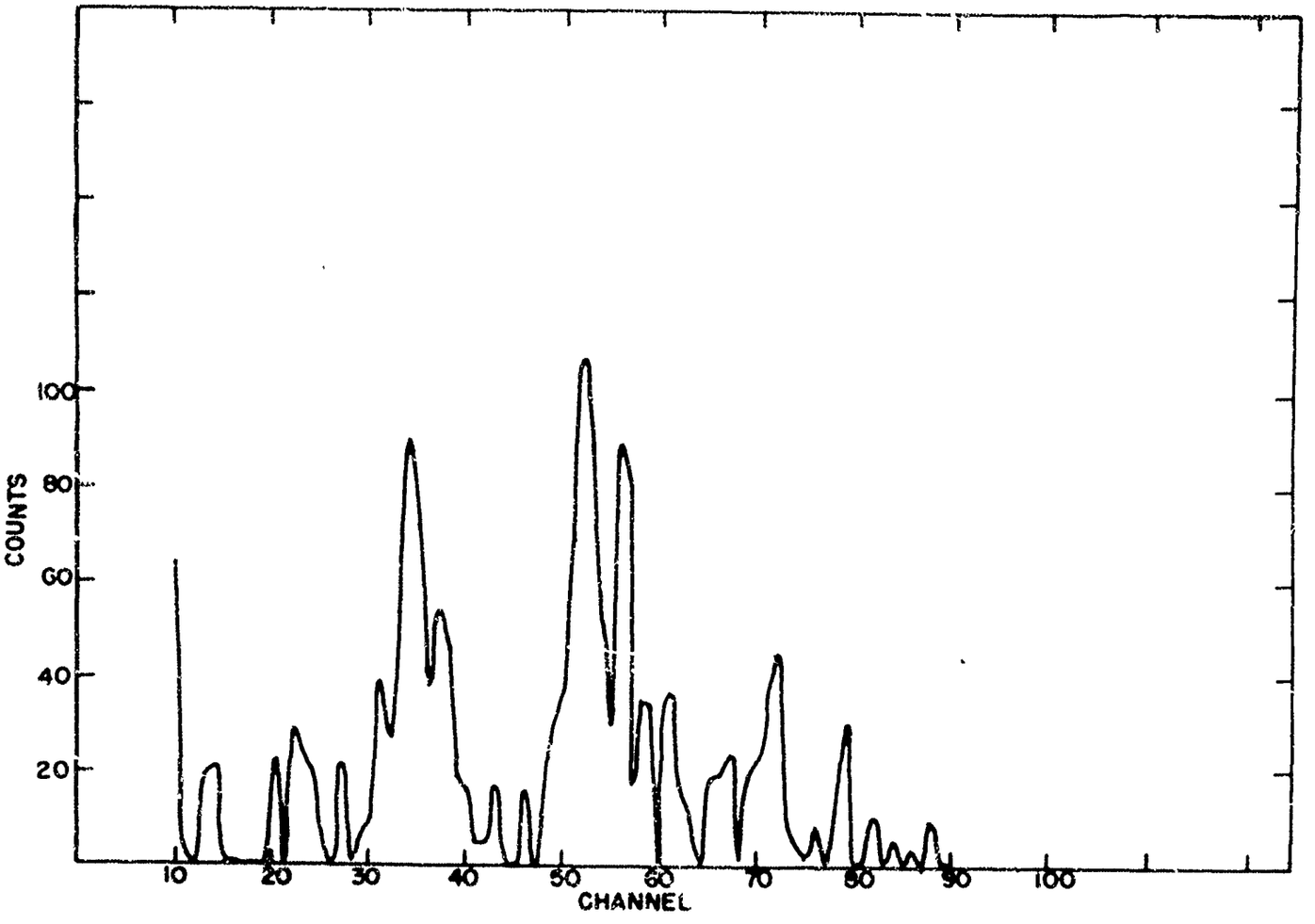

Fig. 9.5—Energy spectrum showing difference between Figs. 9.3 and 9.4. Cells minus precipitate; 9-min run, June 14 and 15, 1955.

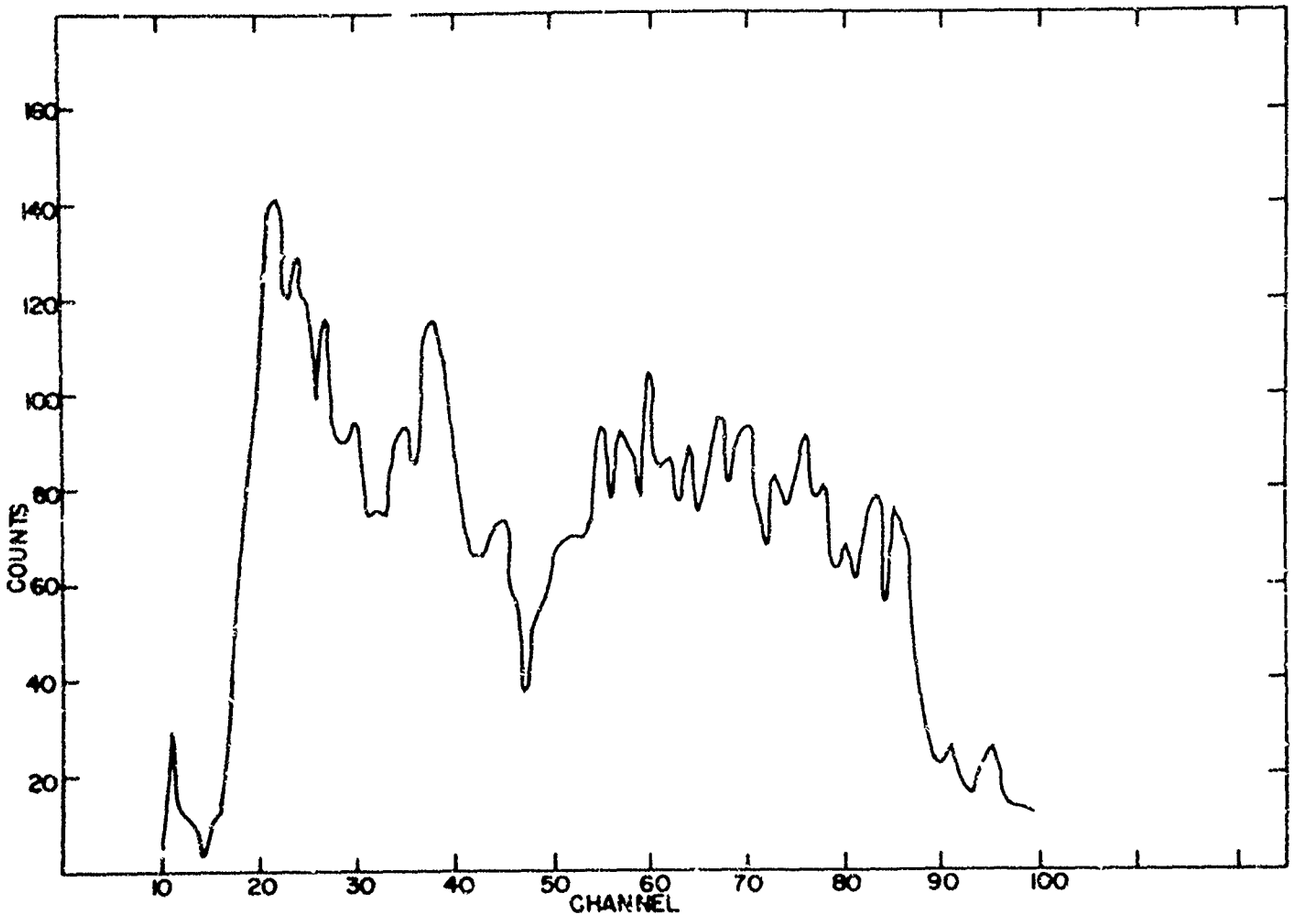

Fig. 9.6—Energy spectrum of precipitate formed 40 hr after experiment started. Fifty per cent radioactive sea water; precipitate only; 7-min run at 0630, June 4, 1955.

In his sample E Cx-2, he isolated from the test area two centric diatoms (Coscinodiscus). These cells had 200 counts/min on May 18, 1955, and 51 counts/min on May 24, 1955. Thus the activity per cell of a diatom cell only three to four times larger than Gonyaulax was one to two orders of magnitude higher than the cells of our experiment. However, the concentration factor may be roughly similar since the activity of the sea water from which these cells were isolated was about one order of magnitude greater.

The lack of soil extract would presumably limit growth. However, growth could also have been limited by radiation damage to the cells. The activity counted was largely due to β emissions, and the total amount of ionizing radiation could have been much higher if appreciable amounts of γ sources were present. However, no data obtained in this work bear directly on the possibility of radiation damage to the phytoplankton.

CHAPTER 10

ELEMENTAL COMPOSITION OF SOME PELAGIC FISHES

By Edward D. Goldberg

10.1 INTRODUCTION

In order to evaluate potential sites of concentration of the various fission products within fishes of commercial importance, a preliminary survey of the elemental composition of a few representative examples was undertaken. The previous work of this type is well summarized in "The Elementary Chemical Composition of Marine Organisms" by A. P. Vinogradov (English translation published by the Sears Foundation for Marine Research, Memoir Number II, New Haven, 1953, 647 pp.). That study suffers from a number of inadequacies: First, it is a compilation of analyses of elements in many different samples but contains few gross analyses of individuals. Second, many of the results were obtained by questionable techniques of the pioneer workers in this phase of biogeochemistry. Finally, there is no critical evaluation of the results in line with modern chemical theory.

Because of the limited time and resources for our investigation, it was initially realized that a small number of results would at best give only gross patterns of elemental distributions. Thus the interpretation of our results must be viewed with the following considerations held firmly in mind:

1. The analyses were made by emission spectroscopy (American Spectrochemical Laboratories, San Francisco, Calif.). Inasmuch as standards could not be prepared for all the matrices encountered in ashed samples of the components that make up a fish, the absolute concentrations, especially for elements whose abundances are less than 0.1 per cent, should be as accurate only within a factor of 2 or 3.

2. The representativeness of the few samples is open to doubt. How much the abundances of the different elements in different parts of a fish depend upon age of the specimen, feeding habits, speciation, etc., is poorly understood. Further, we have no data on such statistical variation among specimens from a population of like size, age, etc.

The samples of fish were obtained in the frozen condition and, following dissection into components, were dried overnight at 100°C. The material was subsequently ashed in Vycor type ovenware. The results of spectroscopic analyses of ashed samples are given in Table 10.1. The only fish whose complete body was assayed in component form is the yellowfin tuna Neothunnus macropterus. Only samples of the flesh and bone were utilized in the analysis of the albacore. There are also five analyses of whole fish, three tunas and two Peruvian anchovies (Engraulis ringens).

The following observations from the data are made not only with respect to their relevance to fission-product assimilation (a restricted spectrum of elements) but also to the more general problems of the disposal of radioactive wastes in pelagic environments.

Table 10.1 —RESULTS OF SPECTROSCOPIC ANALYSES OF ASHED SAMPLES

[illegible] Length: 30 cm. Co. 2407-4-26 collected: 12 August 1954
Location: 28° 49'N, 118° 34'W SS 4805

Sample	Wet Weight	Dry Weight	Ash	Ash	Ca	P_2O_5	Mg	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	Mn	Na	K_2O	Ni	Co	Cr	Cu	V_2O_5	Zn	Ag	Pb	Sr	Ba	Other
Heart and ventricle muscle	34.2380	9.8998	[illegible]	27.	1.8	39.	2.3	2.6	.021	1.4	.006	.066	.0006	.33	.0000	—	.000	.0006	.013	—	.005	—	—	—	.20	Det.01"
Spleen	7.081	2.058	28.5	26.	.95	30.	3.4	10.	.076	2.7	.012	.017	.0005	.75	.0012	.0042	.000	.0035	.006	—	.19	—	.0002	3.5	
Gall bladder	3.2260	0.5895	14.3	15.	3.2	30.	35.	1.9	.04	.20	.030	.000	.0006	.059	.003	.24	.000	.001	.012	—	.00	—	.0007	.14	
Liver	136.4260	33.9996	17.5	25.	.00	51.	2.7	1.3	.13	.95	.012	.013	.0000	.00	.004	.001	.000	.0006	.006	—	.10	.003	.003	.78	Ca. .003 Mn. .30
Pyloric Cecum	139.0	39.05	28.3	33.	3.4	39.	6.0	.78	.010	.26	.014	.010	.0003	.33	.0006	.0009	.030	.0006	.009	—	.012	—	.0007	.14	Det.01"
Stomach	144.2	32.0	18.	40.	1.7	33.	4.0	.00	.007	.98	.002	.006	.0006	.043	.0026	.0017	.013	.0003	.006	—	—	—	.0000	.14	Det.01"
Gill filaments	323.0	32.4	5.6	4.2	30.	45.	4.2	.01	.006	.31	.006	.20	.0017	.026	.0005	.0007	.000	—	.009	—	—	—	—	.05	
Hyoid arch bone	127 1	31.4	5.0	3.7	42.	48.	2.2	.009	.002	.15	.003	.17	.0000	.010	.0013	.0011	.001	—	.006	—	—	—	—	.16	
Epithelia	115.0	26.26	19.	6.0	41.	31.	3.0	.01	.020	.16	.009	.10	.0006	.010	.0011	—	.0009	—	.000	—	—	—	—	.15	Det.01"
Integument	91.2	16.98	2.8	3.2	48.	44.	1.6	.008	.005	.00	.000	.13	.0006	.007	.0012	—	.0013	—	.002	—	—	—	—	.07	
Operculum bone	66.7	26.5	3.5	2.1	48.	47.	1.1	.011	.002	.004	.009	.13	.0003	.006	.0017	—	.001	—	.009	—	—	—	—	.16	
Dorsal fibers	131.9	17.2	6.5	40.	1.4	38.	6.5	.07	.020	.12	.012	.013	.0009	.13	.0006	—	.0009	.0000	.015	—	—	—	—	.12	Det.01"
Skeletal fibers	201.5	38.05	8.7	43.	.99	46.	6.7	.11	.007	.79	.000	.010	.0006	.11	.001	—	.003	.0006	.002	—	—	—	—	.15	Det.01"
Bone	10.6	4.30	3.1	1.9	48.	46.	1.5	.01	.005	.03	.005	.15	.0006	.014	.0016	—	.0000	—	.016	—	—	—	—	.07	
Bone	62.2	30.9	3.2	1.9	50.	43.	1.4	.01	.001	.007	.006	.15	.0013	.010	.0006	—	.0000	—	.000	—	—	—	.0002	.05	

YELLOW FIN TUNA Southwestern Pacific Ocean 30 September 1954
36° 30' S 112° W

Blood sample	12.368	—	46.	13.	0.4	25.	7.0	.12	.093	.00	.006	.003	.0003	.013	.001	.0043	.000	.0009	.012	.015	—	—	.0006	.10	Det.01"
Bone	43.36	27.62	2.5	.53	58.	44.	1.0	.01	.009	.03	.005	.17	.001	—	.0012	—	.0012	—	.009	—	—	—	—	.00	
Pyloric cecum	42.42	9.36	41.	15.3	2.7	33.	5.0	2.1	.04	.32	.005	.017	.0015	—	.0013	.000	.004	.012	.015	—	.013	—	—	.07	
Epithelia	9.39	1.63	70.	6.9	7.0	9.0	7.0	.15	.002	.17	.002	.03	.001	.005	.0009	—	.000	.0007	.020	—	—	—	—	.25	Det.01"
Gills flesh	399.	93.	38.	29.	2.4	37.	4.7	.07	.005	.11	.009	.017	.0008	—	.0009	—	.004	.0012	.000	—	—	—	—	.14	Det.01"
Dark flesh	126.9	36.7	10.3	26.	1.6	34.	4.2	11.5	.009	.99	.012	.019	.0000	.20	.001	.013	.000	.0007	.015	—	.01	—	—	.11	Det.01"
Gallbladder and Chromate	13.4	11.8	5.9	1.9	43.	43.	3.2	.11	.000	.10	.013	.05	.000	.006	.0030	—	.004	.0001	.006	—	—	—	.0001	.12	
Heart	6.41	1.39	28.	14.	0.4	35.	10.	.9	.00	1.4	.000	.000	.005	.013	.004	.0005	.12	.0000	.039	.000	—	—	.0001	.20	
Intestine	15.00	3.47	24.	20.	6.3	28.	10.2	.07	.005	.20	.004	.003	.0013	—	.0015	.0017	.12	.0004	.000	—	—	—	—	.05	
Intestine contents	12.01	2.0	16.	10.2	10.4	23.	35.	.020	.000	.056	.000	.000	.0000	.000	.0000	—	.00	—	.005	—	—	—	—	.03	
Liver	23.6	6.11	26.	27.	1.3	36.	3.4	1.1	.01	2.3	.006	.004	.0012	—	.005	.0029	.004	—	.004	—	—	.007	.0005	.77	
Integument	14.7	5.3	36.	6.6	35.	25.	6.0	.01	.007	.15	.012	.009	.0010	—	.002	.0017	.004	.0006	.012	.017	—	—	.0003	.37	Det.Co" 04 .15
Spleen	2.02	0.52	12.	16.	1.3	46.	20.	.06	.20	3.0	.007	.000	.005	.030	.000	—	.030	.0001	.005	—	—	—	—	.31	
Stomach	24.3	5.02	20.5	11.5	1.5	27.	3.0	20.	.12	.20	.000	.012	.004	1.5	.0004	.005	.130	.0005	.000	—	.27	—	—	1.04	
Stomach contents (2 cupfuls & 1 anchovies)	43.1	10.12	24.	17.	24.	20.	5.2	.20	0.050	.00	.007	.010	.0010	—	.0012	—	.040	.0005	.014	—	—	—	.0001	.39	Det.12"
YELLOWFIN TUNA																									
Whole animal	169.	149.	6.3	27.	32.	41.	4.7	.14	.025	.00	.005	.001	.0003	.00	.0010	—	.0000	.0004	.010	—	—	—	—	.09	
CLIMAX TUNA Subtropical Atlantic																									
Whole animal	1000.		16.	2.6	29.	42.	6.2	.09	.13	.14	.000	.010	.0000	—	.001	.0012	.0007	.0000	.007	—	—	—	.0001	.05	Det. 01"
YELLOWFIN TUNA																									
Whole animal	1200.		16.3	6.1	30.	30.	5.6	.09	.15	.13	.013	.007	.0011	—	.0008	.0009	.013	.0000	.005	—	—	—	.0000	.09	Det.01"
ALBACORE (Northern Pacific)																									
Whole animal	30.0	6.572	3.7	7.7	30.	42.	2.1	5.1	1.7	.08	n/a	.009	.004	.003	.003	—	.0003	.0011	.000	.004	—	—	—	.06	
ALBACORE Thunnalia alalunga (Northern Pacific)																									
Whole animal	37.0	7.398	4.3	6.7	30.	34.	2.3	7.2	2.4	.09	.07	.09	.011	.008	.002	—	.000	.0001	.001	.000	—	—	—	.09	

10.2 ALKALIS

The general pattern of K dominating over Na in fish by factors of 3 or 4 as noted by Vinogradov apparently is confirmed by these analyses. The two tunas that deviate remain anomalous.

10.3 ALKALINE EARTHS

The CaO/SrO ratio in sea water is approximately 480, and within experimental error this result is reproduced in the whole fishes analyzed. Nevertheless, the ratio exhibits much smaller values in the flesh, spleen, liver, stomach, and heart and compensating higher values in the bone, blood, and epidermis (Table 10.2). Apparently the metabolic paths of Ca and Sr

Table 10.2—CaO/SrO RATIO IN YELLOWFIN TUNA, Neothunnus macropterus

Fish Section	CaO/SrO
Spleen	38
Dark Flesh	85
Liver	110
Stomach	120
Heart	130
White Flesh	140
Pyloric Caeca	160
Intestine contents	180
Gill arches and filaments	185
Eyeballs	235
Skin	240
Intestines	270
Bone	295
Blood	370

show strong dissimilarities. The sites of concentration of Sr are the components containing skeletal features (bone, gill arches and filaments, and skin) (Table 10.3). Barium, very curiously, does not show a covariance with strontium. Possibly its metabolism may be related to the sulfate concentrations in the organs of the fish. The strikingly high Mg concentration in the spleen, heart, and gall bladder (or contents), as noted in Table 10.1, invites further investigation rather than comment.

10.4 METALS

The noteworthy abundance of Zn in marine fishes has been known for over 30 years (Vinogradov, page 523), yet its physiological role remains obscure. Zinc is accumulated most obviously in the spleen, liver, stomach, intestines, pyloric caeca, and gall bladder. High radioactive Zn contents (Zn^{65}) have been noted by the Japanese in their studies on contaminated fishes in the Pacific Ocean [Masamichi Saiki, Shinji Okano, and Takajiro Mori, Studies on the

Table 10.3 — DISTRIBUTION OF ZnO AND SrO IN YELLOWFIN TUNA, Neothunnus macropterus

Fish Section	Wt. of Section Grams	% ZnO	Grams ZnO	% SrO	Grams SrO
Blood	12.25	0.18	0.022	0.023	0.0028
Bone	45.16	0.08	0.036	0.17	0.0768
Pyloric Caeca	42.42	0.87	0.369	0.017	0.0072
Eyeballs	8.50	0.25	0.021	0.03	0.0026
White flesh	359.	0.14	0.503	0.017	0.0610
Dark flesh	124.5	0.11	0.137	0.019	0.0237
Gill arches and filaments	53.4	0.12	0.064	0.26	0.1388
Heart	6.41	0.22	0.014	0.018	0.0012
Intestines	15.80	0.86	0.136	0.023	0.0036
Intestine contents	12.81	0.23	0.030	0.059	0.0076
Liver	23.6	0.77	0.182	0.014	0.0033
Epidermis	14.7	0.57	0.085	0.088	0.0129
Spleen	2.02	0.31	0.063	0.035	0.0007
Stomach	24.3	1.04	0.104	0.012	0.0029
Stomach contents (2 squid, 4-½" x 5-½"; 1 anchovy 6")	43.1	0.39	0.039	0.018	0.0078
	787.97		2.08		0.35
% in whole fish		0.26		0.045	

Radioactive Material in the Radiologically Contaminated Fishes Caught at the Pacific Ocean in 1954, Bull. Japan. Soc. Sci. Fisheries, 20: 902-906 (1955)].

The metals Cu, Fe, and Mn show, in general, a pattern similar to that of Zn, showing the highest accumulation in the internal organs. Iron shows strong concentrations in the blood and and circulatory system. The metals further concentrate in the darker flesh over the lighter flesh.

Of interest is the rather strong occurrence of Cd in the liver of the albacore and integument of the tuna. Cadmium has a chemistry quite parallel to that of Zn, and cadmium has never been detected in sea water.

The highest concentration of Ag was found in both fishes in the liver.

10.5 CONCLUSION

In conclusion, the following inferences may be drawn from the data, assuming that the fission-product (or waste-product) element under consideration is assimilated by the fish under the same conditions and in the same chemical forms as in its normal functionings:

1. Elements existing most probably as cationic species in sea water (Mn, Cu, Ni, Zn, etc.) and which tend to form strong organic complexes (see Edward D. Goldberg, Treatise of Marine Ecology, edited by Joel Hedgepeth, GSA, 1957) tend to concentrate in internal organs.

2. The alkaline earths Ca and Sr concentrate in the hard parts, and Sr appears more strongly in the flesh relative to Ca. Surface detection of Sr appears feasible inasmuch as, in both fish analyzed, high abundances of this element were found in the integument.

3. The transition elements (Zr, Ti, and V) are found most abundantly in the internal organs except the heart, flesh, and skin and are found in least quantities in the hard parts.

4. From the known chemistry of Rb and Cs, as well as some studies on plants (R. Scott, A Study of Caesium Accumulation by Marine Algae, Proceedings of Second Radioisotope Conference, Vol. I, pages 373-380, Academic Press, Inc., 1954), one might expect these two elements to be at least as strongly fractionated by the organism relative to Na as is K.

CHAPTER 11

UPTAKE AND ASSIMILATION OF RADIOSTRONTIUM BY PACIFIC MACKEREL

By DeCourcey Martin and Edward D. Goldberg

The problem of the retention of radioactive waste products introduced into marine waters through nuclear detonations by marine organisms has received scant attention. Inasmuch as many fishes and seaweeds are used directly or indirectly in foods, it is of interest to investigate the uptake and assimilation of potentially dangerous fission products by the marine biosphere. This present investigation concerns the uptake of radiostrontium by the Pacific mackerel (Pneumatophorus diego). Radiostrontium is of significance for two reasons: its high fission yield (Sr^{90}) and its long half life (19.9 years). The Pacific mackerel is an important food fish, is closely related to tuna, and is readily adaptable, because of size, to laboratory experimentation.

11.1 EXPERIMENTAL PROCEDURES

11.1.1 Tracer Preparation

Strontium exists as one of the minor constituents of sea water, with a concentration of 7 mg per kilogram of sea water. The isotope of Sr employed, Sr^{90}, has a half life of 19.9 years and emits a beta particle of 0.61 Mev to form Y^{90}. Y^{90} in turn decays by emission of a 2.35-Mev beta particle with a half life of 65 hr to form stable Zr^{90}.

The radiostrontium was obtained in solution as carrier-free $Sr(NO_3)_2$ from the Oak Ridge National Laboratory with an activity of 11.36 mc/ml. The original solution was diluted in acidified distilled water to produce a working solution of approximately 1 μc/μl (1 mc/λ). A 50-λ (0.05-ml) portion was pipetted into gelatin capsules which had been coated with butter to prevent dissolution before use. The working solution was assayed during each feeding period. The average beta activity of five different assays gave 70,630,000 counts/min/50 λ. This is the amount fed to the mackerel.

11.1.2 Inoculation of Fish

Pacific mackerel were chosen as a representative food fish for this study. This fish is readily available, is adaptable to captivity, and is of a convenient size for laboratory work. Six dozen fish of similar age, size, and weight were caught in waters near the shore off La Jolla, Calif. These fish were retained and fed in a large aquarium for several weeks before use.

A 50-λ portion of Sr^{90} in butter-coated gelatin capsules was forced down the throat of each of 24 fish. Ten fish were removed from the retaining aquarium in lots of five and were placed in a tank filled with about 40 liters of sea water. Tertiary amyl alcohol was added until the fish were narcotized. The fish were then removed from the water. A gelatin capsule was forced down the throat of each fish with a glass plunger. The fish were then placed in an experimental tank of 2900 liters of circulating sea water. They recovered from the effects of the alcohol in 5 to 7 min.

The fish failed to eat for several days after the narcotization. Most of them showed signs of diarrhea.

The remaining 14 fish, at a later date, were forcibly fed gelatin capsules by hand. They were then transferred to a second experimental tank within 15 sec. These fish suffered no visible ill effects and voraciously ate bits of chopped salmon within 15 min of the feeding of the radioactive capsule. Although two methods of feeding were used, there appeared to be no significant variations in the results of the radiostrontium uptake and assimilation for the two series of fish.

Following the administration of radiostrontium, the fish were maintained in large tanks with a constant supply of sea water and food. The effluent water from the tanks was diluted and discharged into the ocean. The area around the discharge was monitored hourly for traces of radioactivity for a period of 8 hr following the feeding. It was checked periodically for three weeks thereafter. No appreciable radioactivity was found in the diluted discharge or on the beach sand. The water in the 2900-liter tanks was also monitored and, after feeding five fish, was found to contain 54.2 counts/min/10 ml after 30 min, 52.8 counts/min/10 ml after 1 hr, 45.5 counts/min/10 ml after 2 hr, 24.3 counts/min/10 ml after 4 hr, 15.2 counts/min/10 ml after 6 hr, and 7.7 counts/min/10 ml after 24 hr.

11.1.3 Preparation of Samples for Assay

The fish were sacrificed at regular intervals after administration of the radiostrontium. In several cases more than one fish was killed at a time. The intervals were 15 min; 1, 2, 3½, 4, 11, and 24 hr; and 2, 3, 4, 8, 16, 32, 64, 128, 185, and 235 days. On the fourth and seventh days, fish were found dead and were assayed only for total activity per fish. No significantly greater differences in the total activity per fish were found between the dead fish and a sacrificed fish of the same day than between two fish killed at the same time.

The fish were dissected, and the major organs were removed for assay. A section of the epidermis consisting of about two-thirds of the total was removed. An equal amount of the dermis was assayed. A section of the muscle consisting of from one-third to one-half of the total flesh was separated from all bones and other organs. This was assayed as the "choice" flesh as shown in Fig. 11.5. The remainder of the flesh containing some bone, skin, scales, blood, and bits of other organs was assayed as "waste flesh" in order to determine the total activity in the entire fish from the sum of activity in the organs. The entire gills, heart, spleen, ovaries, kidney, liver, pyloric caeca, small intestine, stomach, and gall bladder were removed. The backbone was separated and picked free of flesh. The head, including the eyes and operculum, was counted separately. Samples of the blood, scales, and fins were also measured.

The above-mentioned organs and tissues were weighed wet, dried at 110°C for three days, weighed dry, and digested in concentrated HNO_3. $HClO_4$ was added, and the mixture was heated until the organic matter was completely digested. The solution was evaporated to dryness. Several samples were lost at this stage owing to ignition of incompletely digested organic matter in perchloric acid. The dried salts were dissolved in water and HCl, diluted to a minimum volume, and stored for future counting. A number of live whole fish were digested, diluted, and prepared for counting in the same fashion.

11.1.4 Method of Assay of Radiostrontium

The beta particles from the Sr^{90} and Y^{90} in the equilibrium mixture have maximum energies of 0.61 and 2.35 Mev, respectively, and hence are easily detectable. Seven to eight days was allowed between the sacrifice and beta counting of the fish in order to permit the 65h Y^{90} to reach equilibrium with the Sr^{90}.

The solution of ashed organs was counted in 0.2-ml aliquots on 1-in.-diameter copper planchets mounted on cardboard disks and covered with one layer of scotch tape. A scintillation counter consisting of a plastic p-terphenyl crystal coupled to an RCA 5819 photomultiplier tube was used in conjunction with a conventional scaler. The planchets to be counted were mounted 2 mm below the p-terphenyl phosphor in a shield of ½-in. plastic surrounded by 2 in. of lead.

The self-absorption of the small amount of salt was taken into account in the following way: Appropriate amounts of ash from a control fish were added to the standard solutions, and the samples were then assayed.

The planchets were each counted at two or more different periods to check for any build-up or decay of Y. Only in the case of the stomach and intestines of the 1, 2, and 3½-hr fish was any increase in activity noted. The maximum increase observed was in the stomach of the 3½-hr fish, which showed an increase of 10 per cent over two weeks' time.

11.2 RESULTS

The results obtained from assaying the radiostrontium content of the different tissues and organs are expressed as the percentage per organ and as the percentage per gram of dry weight. The sum of the activities of all components was calculated to give the total activity per fish. This result is presented as the percentage of the original dose remaining in the fish. As checks on these results, the solutions of the organs were added together to produce a solution of the entire fish. Furthermore, 10 fish were dissolved whole and counted as individuals.

The excretion of radiostrontium by the Pacific mackerel is shown in Fig. 11.1. The percentage of the initial dose remaining in the fish is plotted against time. Half of the activity was lost between 12 hr and one day. After one or two days the level of activity remained almost constant. This indicates that about 1 to 5 per cent of the ingested radiostrontium may be expected to remain in the fish for a long period of time.

Figure 11.2 shows the activity remaining in the skeleton of the fish. After one day, almost 80 per cent of the radiostrontium was lodged in the skeleton. This value remained constant thereafter. However, the percentage of activity in the gills decreased after the first day, whereas the percentages in the head and backbone increased.

Figure 11.3 shows the activity found in the total flesh of the fish. The curve is based on the activity per gram of dry flesh as shown in Fig. 11.5. Only samples consisting of from one-third to one-half of the total flesh were assayed. These were boneless fillets. The remaining flesh contained some bone, blood, scales, and body fluids and consequently would have given a higher activity. This waste flesh was about four times as active as the choice flesh.

Figure 11.4 shows the weight deviation of each of 12 fish from the average weight. The weight of each dry organ was multiplied by a correction factor to reduce the fish to the same effective weight. Assuming no variation in relative size of organs with weight of fish, a small fish would be expected to have a greater concentration of activity per gram than a larger fish which had absorbed the same dose. The activity per gram of dry organ was multiplied by (wet weight of the fish)/(average wet weight of all fish).

Figures 11.5 and 11.6 show the concentration of activity in tissue and organs in percentage of retained radiostrontium per gram of dry organ plotted against days since administration of the tracer.

A large percentage of the radiostrontium was found in the gills one day after inoculation. This is the site of extrarenal excretion of salts by the organism, and probably a large percentage of the radiostrontium was excreted by the gills. The feces were not collected. The

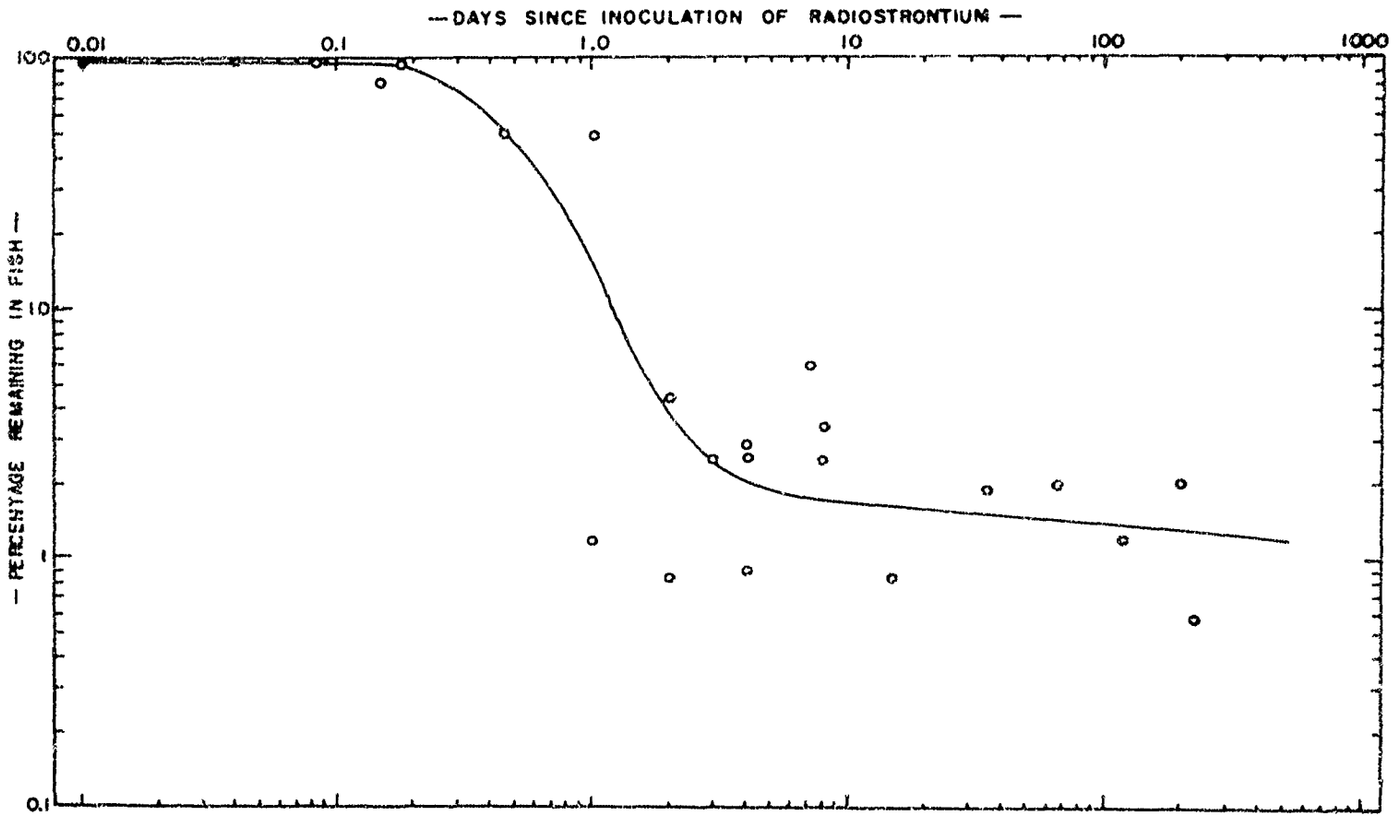

Fig. 11.1 —Uptake of radiostrontium by Pacific mackerel in percentage of dose remaining since inoculation. Each point represents one fish.

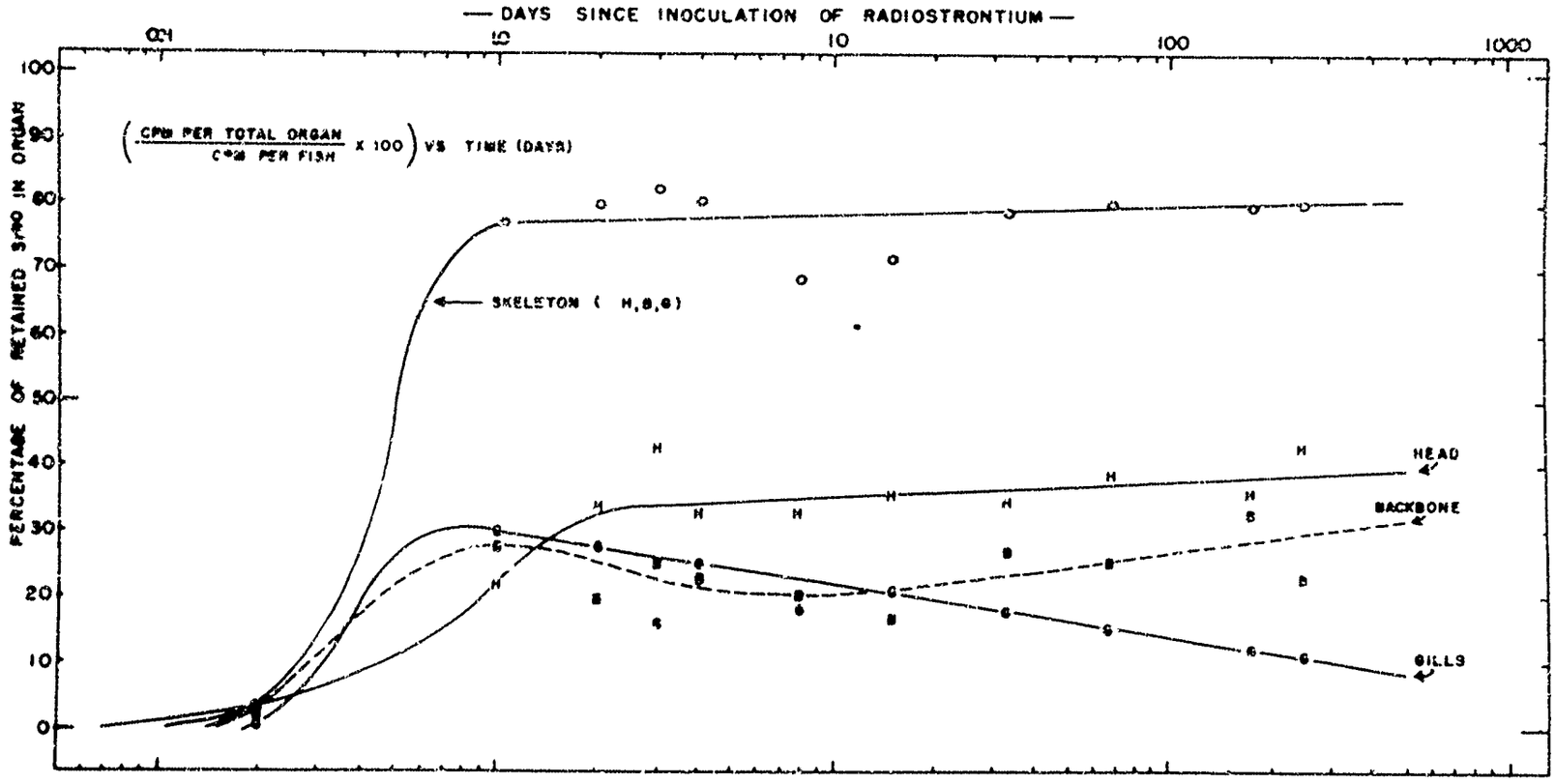

Fig. 11.2—Deposition of radiostrontium in head, backbone, gills, and total skeleton of Pacific mackerel, showing percentage of retained dose in each organ.

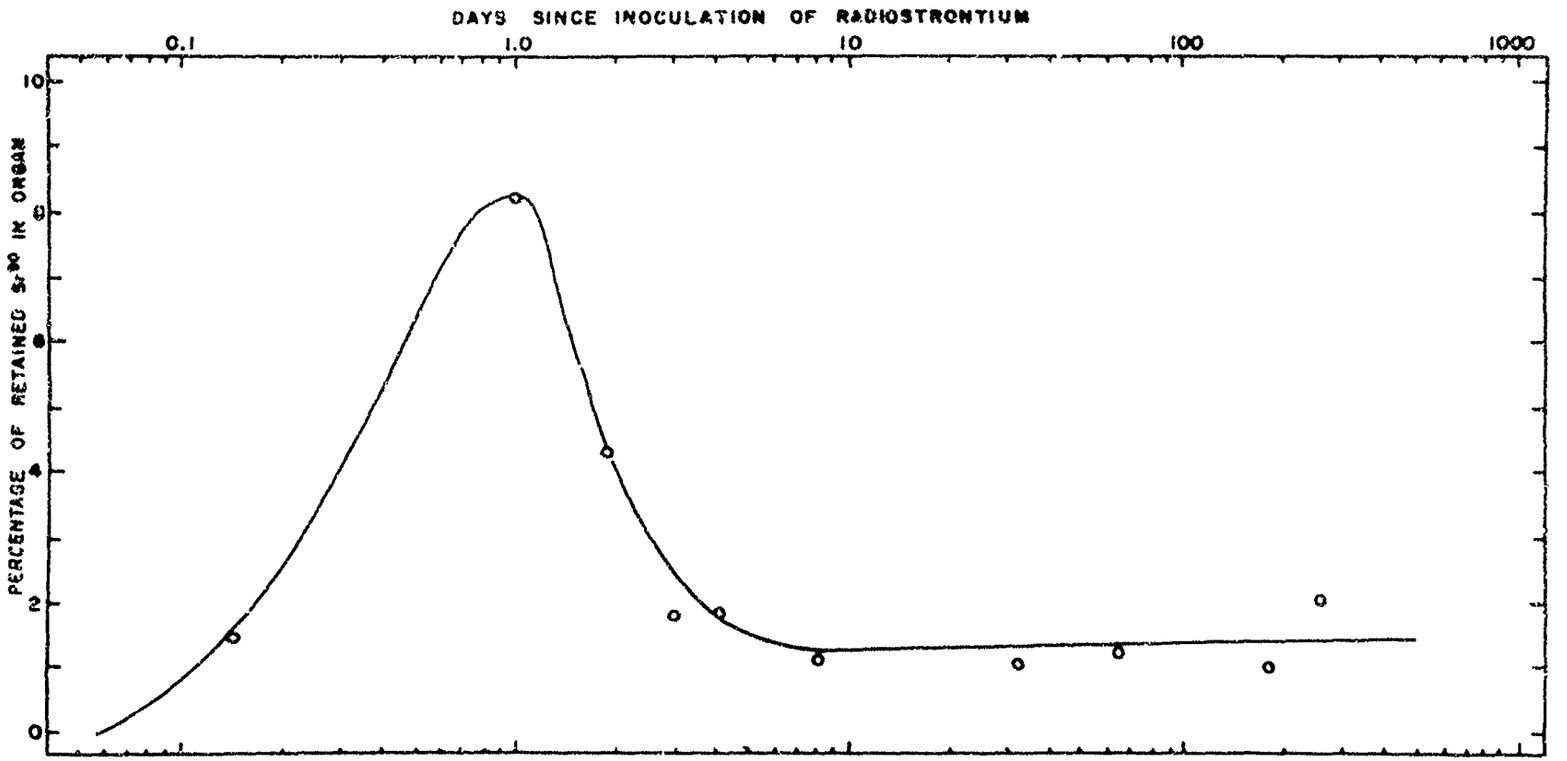

Fig 11.3—Approximate deposition of radiostrontium in total flesh of Pacific mackerel as calculated from choice boneless fillet consisting of an average of one-third of total flesh.

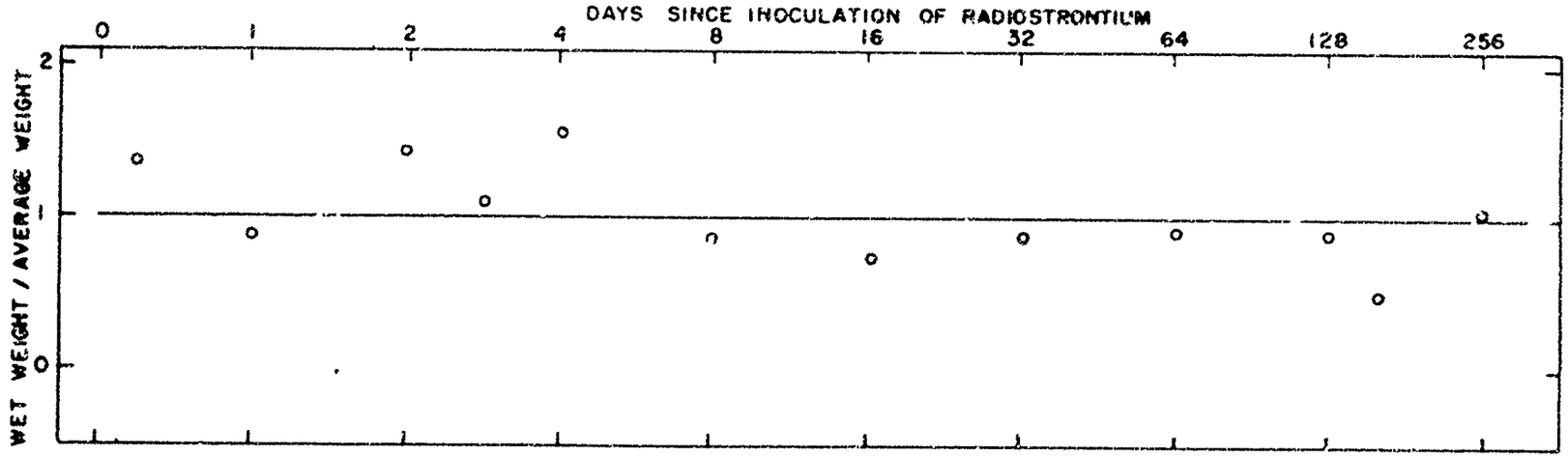

Fig. 11.4—Correction factors applied against weight variation of Pacific mackerel. Each point represents the deviation of the wet weight of a fish from the average wet weight of the 12 fish.

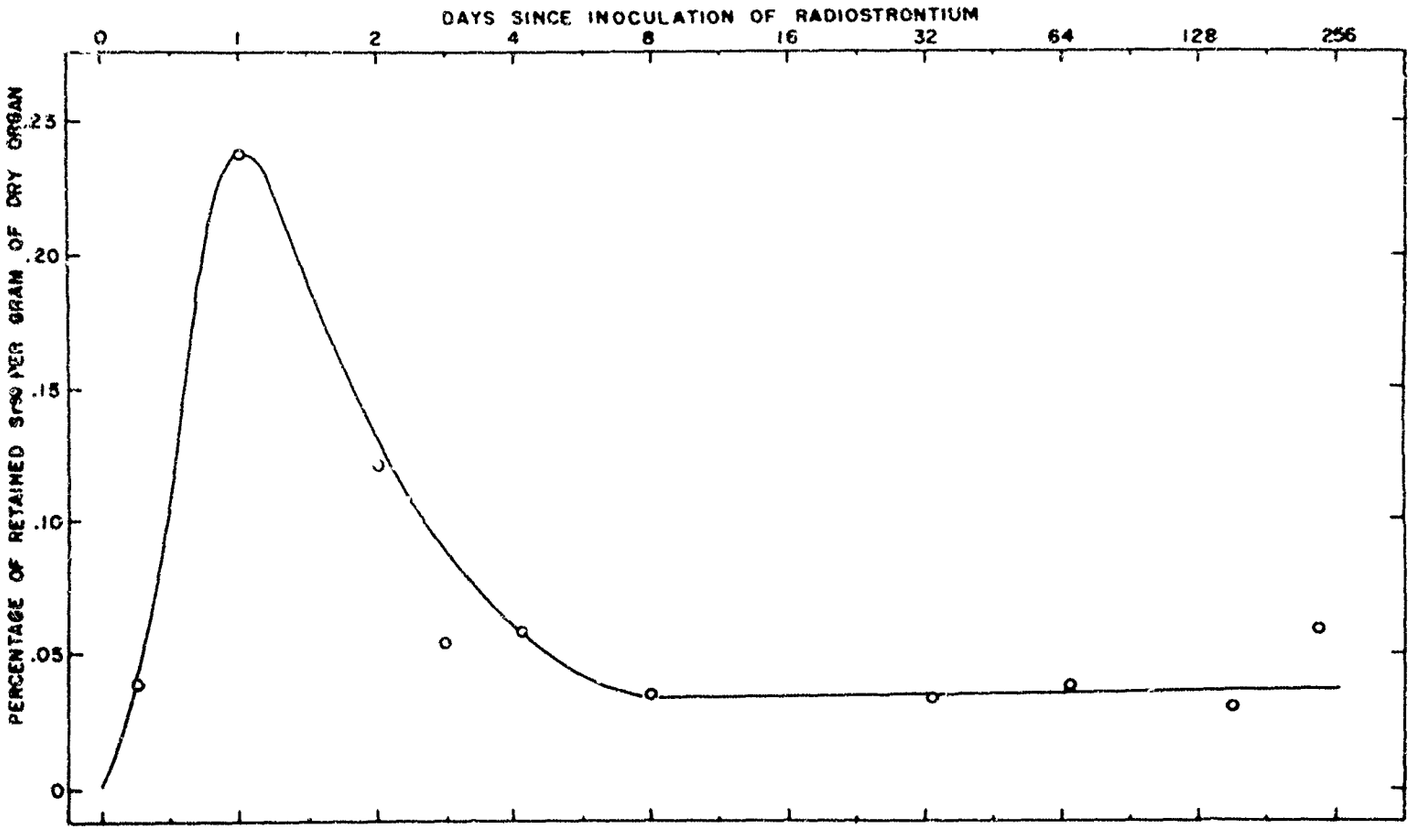

Fig. 11.5—Uptake of radiostrontium by choice flesh of Pacific mackerel in percentage of absorbed strontium per gram of dry organ following oral administration.

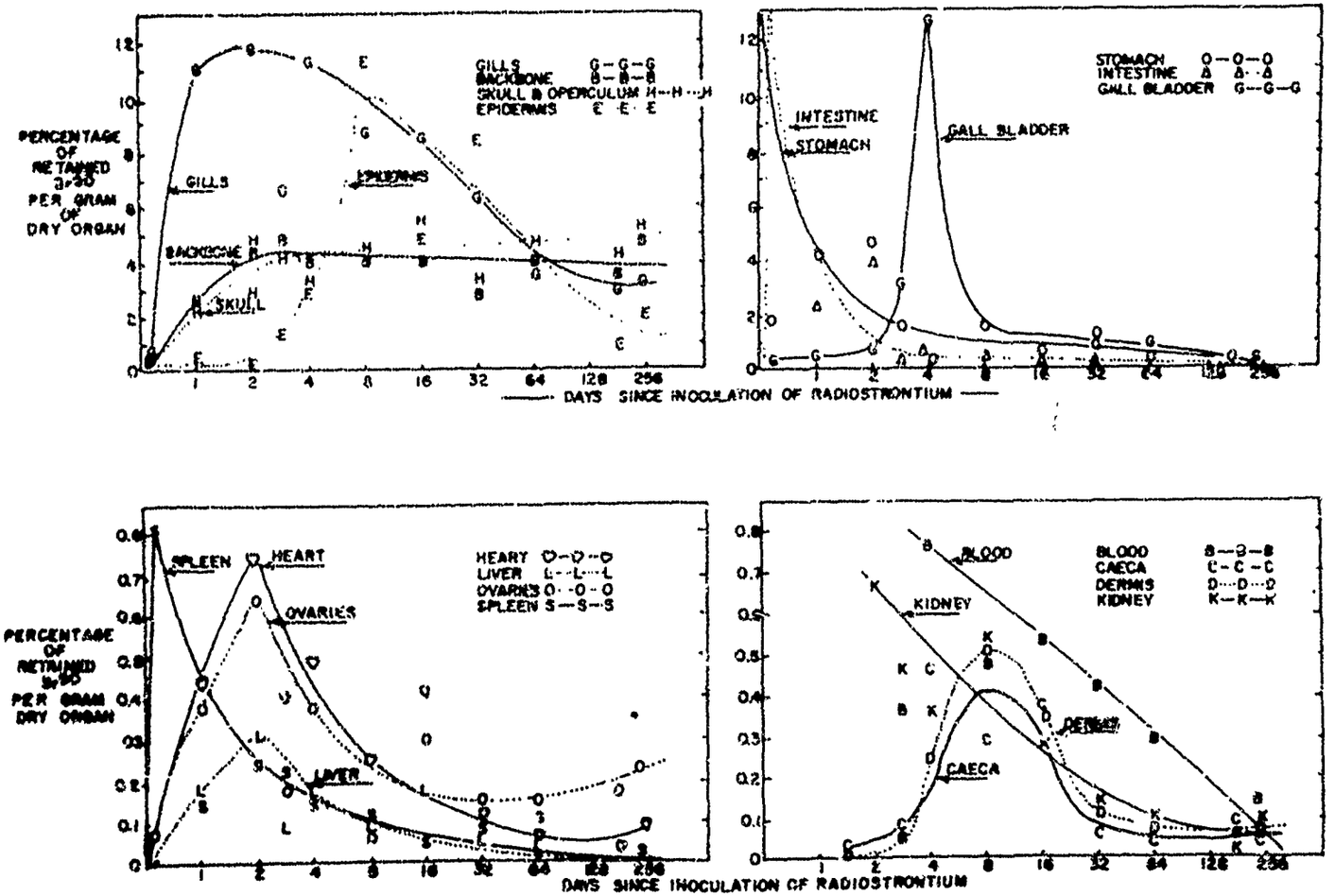

Fig. 11.8—Uptake of radiostrontium by principal organs of Pacific mackerel in percentage of absorbed dose per gram of dry organ following oral administration. The curves are corrected for variations in the weights of the individual fish.

activity in the gills steadily decreased after the initial maximum, whereas the activity in the backbone and head either remained at a constant level or increased with time.

The radiostrontium rapidly passed from the stomach into the small intestine. At the same time, the activity was concentrated by the spleen. As the radiostrontium in the intestines decreased, the gall bladder, heart, liver, and ovaries showed an increase and then a decrease after 2 to 4 days. The activity found in the epidermis, dermis, and pyloric caeca increased in percentage to a maximum on the eighth day and then followed the loss by the gills. Only the head and backbone showed no decrease with time. Apparently the radiostrontium is first absorbed throughout the body; it is then excreted by the gills or is fixed in the bones.

11.3 SUMMARY

Pacific mackerel were fed radiostrontium, which was then measured in various organs over a period of 235 days.

Ninety-five per cent was excreted in 24 hr. The remaining 5 per cent remained fixed in the body throughout the duration of the experiment.

Eighty per cent of the fixed activity was located in the calcareous portions of the fish.

The gills showed the greatest activity per unit weight in one to three days after feeding, indicating that the radiostrontium was being excreted by these organs during this period.

After two days the flesh showed little activity per gram of fish.

CHAPTER 12

FIELD STUDIES OF UPTAKE OF FISSION PRODUCTS BY MARINE ORGANISMS

By Leo Berner, Robert Bieri, Edward D. Goldberg, DeCourcey Martin, and Robert L. Wisner

12.1 INTRODUCTION

The interaction between the members of the marine biosphere and the various chemical components of sea water commands attention as one of the focal points in studies of productivity and sedimentation. For extending our understanding of biogeochemical processes, the permutations of the composition of sea water produced by the detonation of nuclear devices offer great promise. First, the introduction of minute amounts of easily measurable radioactive substances provides ready access to the paths of such substances from the hydrosphere to the organisms and subsequently from organism to organism. Single elements, as well as suites of elements, can readily be studied by already well-established radiochemical techniques for their isolation and quantitative analysis. Second, time studies on the retention of assimilated substances can be undertaken. The biological half lives of elements in marine organisms, in conjunction with the respective concentration factors, are of paramount importance in considerations upon the long-time effects of oceanic nuclear tests.

The purpose of the field studies on Wigwam was to make a broad survey of the distribution of fission products between the marine waters and members of the biosphere. The work was limited in two ways: (1) The time available for the sampling of both water and organisms was secondary to the main objectives of the Scripps Institution of Oceanography vessels, and stations could not be made in every definitive area of interest. (2) Owing to the lack of personnel, equipment, and space aboard the vessels, chemical separations of the fission products were not made. This latter limitation prevented studies on the more intimate biogeochemistry of the various nuclear products from the explosion. Nonetheless, a preliminary picture of the interaction between fission products and marine plants and animals has evolved.

12.2 METHODOLOGY

12.2.1 Sampling Techniques

Samples of sea water were assayed in the filtered (millipore type HA filters, pore size about 0.5 μ) and the unfiltered states. The samples were collected in plastic bottles (Plexiglas) and in brass Nansen bottles, as well as in rubber buckets, from the surface. Samples of the zooplanktonic organisms were collected both in meter nets and in the high-speed plankton

collector. Samples of larger organisms were individually separated and washed first in inactive sea water and then twice in distilled water. The washes were changed extensively and never displayed any measurable contamination. Some samples were frozen immediately after they were obtained, and these were maintained in this state until analysis. All specimens were identified before radioactivity assays were made.

12.3.2 Radioassay Techniques

Activity measurements were made for both beta and gamma particles. The beta activity was measured either with an end-window G-M tube (window thickness 1.4 mg/cm^2) or with a terphenyl scintillation crystal coupled to a Du Mont 6292 photomultiplier tube. The gamma activity was measured with a NaI(Tl) crystal coupled to a Du Mont 6292 photomultiplier tube. Conventional scaling equipment was used.

A single-channel step-pulse-height analyzer (Devtron) was used to obtain the gamma spectrum of samples from 0.3 to 1.5 Mev. The detector was a NaI(Tl) well type crystal coupled to a Du Mont 6292 photomultiplier tube. The spectrum was divided into 100 channels having a channel width of 1 volt. The instrument was standardized routinely with Co^{60}, Zn^{65}, and Cs^{137} sources. Samples were counted over each channel for uniform times ranging from 1 to 15 min.

For beta and gamma assays the sea-water aliquots were evaporated under an infrared lamp and then coated with a lacquer using a bomb type sprayer. The zooplankton samples were initially dried at 110°C in an oven and then coated with the lacquer.

Samples for gamma spectral analyses were placed in small screw-top glass vials which were then completely sprayed with the lacquer. Water samples were placed in glass vials which were closed off by sealings. The vials were washed in clear water before analysis.

The number of organisms or the amount of sea water for assay was approximated by holding the sample near a survey meter (Beckman MX 5) to ascertain a rough value for its activity. No samples showing activities greater than 20,000 counts/min were assayed to avoid coincidence effects. Samples were counted until their activity decreased to a value near background. All samples were counted to a probable error of 10 per cent or better in the net counting rate.

Aluminum, copper, and plastic planchets were used for the beta and gamma measurements. In a single series, all planchets were of the same composition, and therefore ready comparisons can be made. However, neither backscattering nor self-absorption corrections have been applied in any case.

12.3 RESULTS

12.3.1 Water

On May 19, 1955, a deep-water sample was brought aboard, and the distribution of activity between the soluble and particulate states was ascertained by filtering the water through a membrane filter (pore size approximately 0.5 μ) and assaying the activity under a gamma scintillation detector. Approximately 50 per cent of the activity could be removed by the filtration process. This measurement puts an upper limit on the amount of activity in true ionic states. It is probably much too high, considering the data of N. Ballou (personal communication).

Gamma-ray spectra of untreated waters, collected at different stations and at different times, were run daily from May 19, 1955, until the middle of August 1955. These samples showed no gross pattern differences when they were counted at the same time. This result indicates that no measurable fractionation of isotopes took place in these waters through the action of physicochemical or biological processes. In August 1955 the only detectable activities remaining in unfiltered waters were Zr and Ru, as measured by their gamma spectra.

12.3.2 Organisms

Tables 12.1 to 12.9 give the relative activities of organisms collected during the operation. It was apparent quite early in the data analyses that the activity of an organism from a given haul was dependent upon its feeding habits. Thus the organisms are tabulated in groups of feeding types: C, ciliary; M, mucous; S, setal; R, rapacious; P, pseudopodial; and T, tentacular. A survey of these results brings forth a number of generalizations:

1. The ciliary and mucous filterers show the highest accumulations of radioactivity per organism.

2. The setal filterers in general are the second highest accumulators of radioactivity but show also rather strong variations.

3. The rapacious forms such as the calanoid Candacia and Chaetognatha show considerable variation, although less than the setal filterers, and in general possess lower counts per organism.

4. Radiolarians (pseudopodial filterers) have rather high accumulations of activity, whereas a single foraminifera (sample N-1, Table 12.7) showed a zero count in a sample that had few organisms with high activities.

5. The tentacular feeders (coelenterates) had rather low counts per organism. It should be emphasized that the validity of such comparisons rests upon the assumption that the organisms from a given haul were all exposed to the same levels of activity for essentially the same periods of time. Obviously this is not true. Nevertheless, these generalizations can lead us to formulate a general picture of the distribution of activity between the hydrosphere and biosphere.

It is hard to avoid the induction that the dominant form of uptake is that of particulate material. Inasmuch as at least 50 per cent of the activity is in a dispersed solid form, those organisms that produce an absorption sheet (mucus) or a filter (cilia) should be most effective in removing the solid phases from the water. A confirmation of this hypothesis may be found in the comparison of the gamma spectra of the water and of the organisms. In Figs. 12.1 and 12.2 are the curves for pteropod X-1 and water sample 4. The patterns are quite similar for the gross gamma activity. In Figs. 12.3 and 12.4 the comparison is made for June 24, 1955. The patterns again are quite similar, except that the organism does not show the peak at channel 68. Similarly, late in July, the pteropod and water sample (Folsom No. 5) show essentially the same pattern, except that the organism lacks a peak around channel 99 (Figs. 12.5 and 12.6).

Without further knowledge, one may tentatively assign these peaks, missing in the organisms, to chemical species that the organism did not favor in uptake. It is tempting to consider them as soluble species.

Of interest are the pteropods, a ciliary feeding group, that were nearly always possessors of high accumulations of activity. In Table 12.7, samples N-9 and N-10, the partition of activity between the hard and soft parts of the organisms Cavolinia inflexa was made, with the result that the dominant amount of activity is found in the body. The shells of the pteropods are formed of calcium carbonate. A further dissection of the bodies of the organisms was made, and in Cuvierina columnella, taken from net tow 13, the activity was as follows: 1146 counts/min in the liver and gut; 35 counts/min in the shell; and 108 counts/min in the remainder of the soft parts.

The analyses of fishes, given in Tables 12.10 to 12.15, indicate that the majority of the activity could be isolated in the stomach contents, intestines, and associated organs. We cannot say how long the fish had been in the area of contamination. Assuming that they had been there but a short time and that they had been actively feeding, it is understandable that contamination would appear only in the alimentary tract. If, however, they had been present a longer time and had ingested a sufficient amount of contaminated food, then we may expect to find contaminants within the various organs and the body tissues. It is rather apparent from the results that both these events had occurred.

The studies resulting from Operation Wigwam may be regarded as being entirely elementary in nature. Contrary to controlled laboratory experiments, such field studies, or labora-

(Text continues on page 146.)

Table 12.1 — GAMMA ACTIVITY OF ORGANISMS COLLECTED MAY 16, 1955
(Mounted on Aluminum Planchets; Counted May 16, 1955)

Sample No.	No. of Organisms	Feeding Type	Organism	Total Activity	Activity Per Organism
B-1	2	S	Euphausiids (12-15 mm)	46,111	23,055
B-2	2	S	Hyperiids	830	415
B-5	1	S	Mysid	100	100
B-10	1	S	Calanus	3,357	3,357
B-11	1	S	Ostracod	400	400
B-12	1	S	Calanus	3,217	3,217
B-6	1	S?	Decapod Larva	865	865
B-3	1	R	Polychaete	907	907
B-4	2	R	Sagittas (8 mm long)	575	288
B-7	2	R	Candacia aethiopica	519	260
B-8	2	R	Candacia aethiopica	665	333
B-9	1	T	Siphonophore Bract (Diphyes sp.)	820	820
B-13	1	Diatom	Coscinodiscus Rex	50	50

Table 12.2—BETA ACTIVITY OF ORGANISMS COLLECTED MAY 16, 1955
(Mounted on Plastic Planchets; Counted May 18, 1955)

Sample No.	No. of Organisms	Feeding Type	Organism	Total Activity
P-4	3	S	Calanoid	7460
P-5	1	S	Calanus	5155
P-6	1	S	Stylocheiron affine adult	612
P-8	1	S	Euphausiid furtulia	84
P-9	1	S	Ostracod	3807
P-12	1	S	Calanoid	8116
P-13	1	S	Calanoid as P-12	2794
P-14	1	S	Calanoid	807
P-1	1	R	Candacia aethiopica	100
P-2	1	R	Candacia aethiopica	222
P-7	1	R	Tomopteris	14
P-11	1	R	Polychaete	17
P-15	1	R	Sagitta	483
P-3	1	R	Head of Polychaete (See B-3)	61
P-10	1	?	Galatheid larva	3838

Table 12.3 — ORGANISMS AND THEIR GAMMA ACTIVITY FROM HORIZON SAMPLE 2
(Taken from Peaks at Channels 48 to 50 in the Gamma-ray Spectrometer;
Counted May 20, 1955)

Sample No.	No. of Organisms	Feeding Type	Organism	Total Activity	Activity Per Organism
H-1	1	C	Euclio pyramidatus	7262	7252
H-4	1	S	Calanoid Copepod	347	347
H-9	3	S	Calanoid Copepods	313	104
H-17	22	S	Euphausiids (6-15 mm)	531	24
H-18	24	S	Calanoid Copepods (2-3 mm)	1020	42
H-12	17	R	Larval Fish (8-15 mm long)	712	42
H-13	12	R	Sagitta hexaptera (20 mm) (6)		
			Sagitta colinefornica (12 mm) (6)	841	70
H-14	30	R	Candacia aethiopica	1355	45
			Stages 4, 5, 6		
H-15	15	R	Sappharinids	42	3

Table 12.4 — ORGANISMS AND THEIR BETA ACTIVITY FROM HORIZON SAMPLE 2
(Counted May 18, 1955)

Sample No.	No. of Organisms	Feeding Type	Organism	Total Activity	Activity Per Organism
HCu-6	4	S	Euphausiids	3907	977
HCu-7	1	S	Decapod Larva	4359	4359
HCu-8	5	S	Calanoid Copepods	14547	2909
HCu-10	6	S	Amphipods	5973	996
HCu-11	4	S	Ostracods	4867	1217
HCu-3	2	R	Larval Fish	4400	2200
HCu-4	4	R	Sagittas	1965	491
HCu-5	4	R	Candacia aethiopica	4115	1029
HCu-9	6	R	Sappharinids	666	111
HCu-1	1	P	Radiolarian	982	982
HCu-2	2	Diatom	Centric Diatoms	200	100
			Coscinodiscus		

Table 12.5 — ORGANISMS AND THEIR BETA ACTIVITY FROM THE BAIRD CLOSING-NET SERIES
(Haul 9, Taken May 19, 1955, and Counted May 19, 1955)

Sample No.	No. of Organisms	Feeding Type	Organisms	Total Activity	Activity Per Organism
XCu-4	1	M	Salp	5775	5775
XCu-3	1	C	Pteropod	6209	6209
XCu-1	12	S	Euphausiids	1309	199
XCu-6	1	S	Amphipod	5	5
XCu-7	1	S	Ostracod	23	23
XCu-2	1	R	Polychaete	0	0
XCu-5	1	R	Chaetognath	178	178
XCu-8	1	P	Radiolarian	2320	2320

Table 12.6—BETA ACTIVITY OF ORGANISMS FROM NET HAUL OF MAY 22, 1955
(Counted May 23, 1955)

Sample No.	No. Of Organisms	Feeding Type	Organisms	Total Activity	Activity Per Organism
C-15	1	M	Salp	9,918	9,918
C-3	15	C	Cavolinia inflexa	27,895	1,860
C-10	2	C	Styliola subula	17,477	8,739
C-11	1	C	Euclio pyramidatus	11,339	11,339
C-20	1	C	Gymnosomatous	3,447	3,447
C-26	1	C	13 mm. Cuvierina columnella	3,181	3,181
C-28	1	C	Diacria trispinosu	4,148	4,148
C-30	12	C	Diacria quadridentatia	639	53
C-1	3	C	Cyclothone signatu	468	156
C-2	1	S	Thysanopoda aequalis	795	795
C-4	1	S	Hygophum reinhardti	271	271
C-5	5	S	Medtridia atra	76	15
C-6	1	S	Amphipod, 25 mm. long (Oxycephalus)	118	118
C-7	2	S	Amphipods, Phronima, 25 mm.	1,658	829
C-8	5	S	Euphausiids, misc.	919	184
C-12	1	S	Metridia, 8 mm. long	29	29
C-14	1	S	Decapod, 25 mm. long	57	57
C-24	10	S	Calanoids, misc.	1,224	122
C-27	5	S	Ostracods	44	9
C-29	1	S	Calanoid Copepod	75	75
C-13	1	R	Pteratraiechid, 45 mm. long	114	114
C-16	3	R	Sagitta hexaptera, 20 mm.	112	112
C-17	4	R	Sagitta planctonis, 12 mm.	17	4
C-19	3	R	Chaetognath, Surface Living Pterosugita draco	6	2
C-21	3	R	Polychaetes	27	9
C-22	2	R	Squid, 8 mm.	188	94
C-23	4	R	Candacia aethiopica	458	115
C-25	3	R	Sapphirinids	61	20
C-9	4	P	Radiolarians, 8 mm. diameter	587	147
C-18	5	T	Diphyes, Bracts, 15 mm.	118	24

Table 12.7—BETA ACTIVITY OF ORGANISMS FROM NET TOW 13 OF THE BAIRD
(Taken May 23, 1955, from 0 to 500 Meters; Counted June 6, 1955)

Sample No.	No. Of Organisms	Feeding Type	Organisms	Total Activity	Activity Per Organism
N-4	1	C	Cavolinia inflexa, 6 mm.	990	990
N-5	2	C	Limacina, sp. 1.5 and 0.5 mm. long	78	39
N-9	1)	C)	Cavolinia inflexa, 6.5 mm. long	54	54
N-10	1)		Shell above, body below	2,988	2,988
N-11	1	C	Hyperiid Amphipod	182	182
N-2	2	S	Barnacles, Cyprids, 1 and 1.5 mm. long	0	0
N-6	1	S	Euphausiid, 16 mm. long Thysanopoda	112	112
N-7	1	S	Euphausiid, 24 mm. long Thysanopoda	98	98
N-8	1	S	Decapod, 20 mm. long	54	54
N-13	1	S	Ostracod, 8 mm. long	47	47
N-3	1	R	Atlantid, 2.5 mm. max. diam.	27	27
N-12	1	R	Myctophid, 10-11 mm.	132	132
N-1	1	P	Globorotalia truncatulinoid	0	0

Table 12.8—GAMMA ACTIVITY OF ORGANISMS COLLECTED MAY 19, 1955, AT 2300, FROM PLANKTON TOW 10 OF THE BAIRD
(Oblique Tow; Counted May 20, 1955)

Sample No.	No. Of Organisms	Feeding Type	Organism	Total Activity	Activity Per Organism
T-6	3	C	Cavolinia inflexa	651	217
T-7	4	C	Pteropods	20	5
T-8	1	C	Pteropod	16	16
T-2	1	S	Deep Sea Red Shrimp, 20 mm.	23,789	23,789
T-1	5	S	Euphausiids	55	11
T-4	2	S	Copepods, Metridia, 10 mm. black	211	106
T-11	8	S	Copepods, Calanoid, 3 mm. red	178	22
T-12	6	S	Hyperiid Amphipods	0	0
T-14	1	S	Decapod, 15 mm.	10	10
T-18	10	S	Ostracods, 3 species	4	0.4
T-3	1	R	Cyclothone, 25 mm. fish	59	59
T-5	5	R	Misc. Larval Fish	13	3
T-9	1	R	Atlantid	3	3
T-13	1	R	Cyclothone, 15 mm.	10	10
T-12	12	R	Candacia aethiopica	3	0.25
T-17	4	R	Polychaetes	4	1
T-16	4	T	Diphyes Bracts	14	4

Table 12.9—BETA ACTIVITY OF ORGANISMS COLLECTED MAY 21 AND 22, 1955, FROM 2030 TO 0530, ON THE BAIRD BY BAZOOKA NET
(Counted June 6, 1955)

Sample No.	No. Of Organisms	Feeding Type	Organism	Total Activity	Activity Per Organism
Z-3	1	C	Pteropod	27	27
Z-10	2	C	Pteropods	3,072	3,072
Z-22	1	C	Pteropod, Styliola Sp.	20	20
Z-16	1	C	Naked Pteropod	3,264	3,264
Z-1	2	S	Decapods	259	130
Z-2	3	S	Mysid, Ceromysida	129	43
Z-4	3	S	Calanus	337	112
Z-5	3	S	Euphausiids	371	124
Z-7	3	S	Ostracods	5	2
Z-18	3	S	Euphausiids	19	6
Z-19	1	S	Barnacle, Cyprid	26	26
Z-21	1	S	Amphipods	31	31
Z-14	2	S	Amphipods	17	8
Z-6	3	R	Sagitta sp.	64	21
Z-9	4	R	Fish Larvae	78	20
Z-11	1	R	Candacia aethiopica	7	7
Z-12	1	R	Sapphirinids	0	0
Z-15	2	R	Atlantids	0	0
Z-17	1	R	Polychaete	8	8
Z-13	1	P	Colonial Radiolarian Colony	97	97
Z-8	1		Diatom Conscinodiscus		

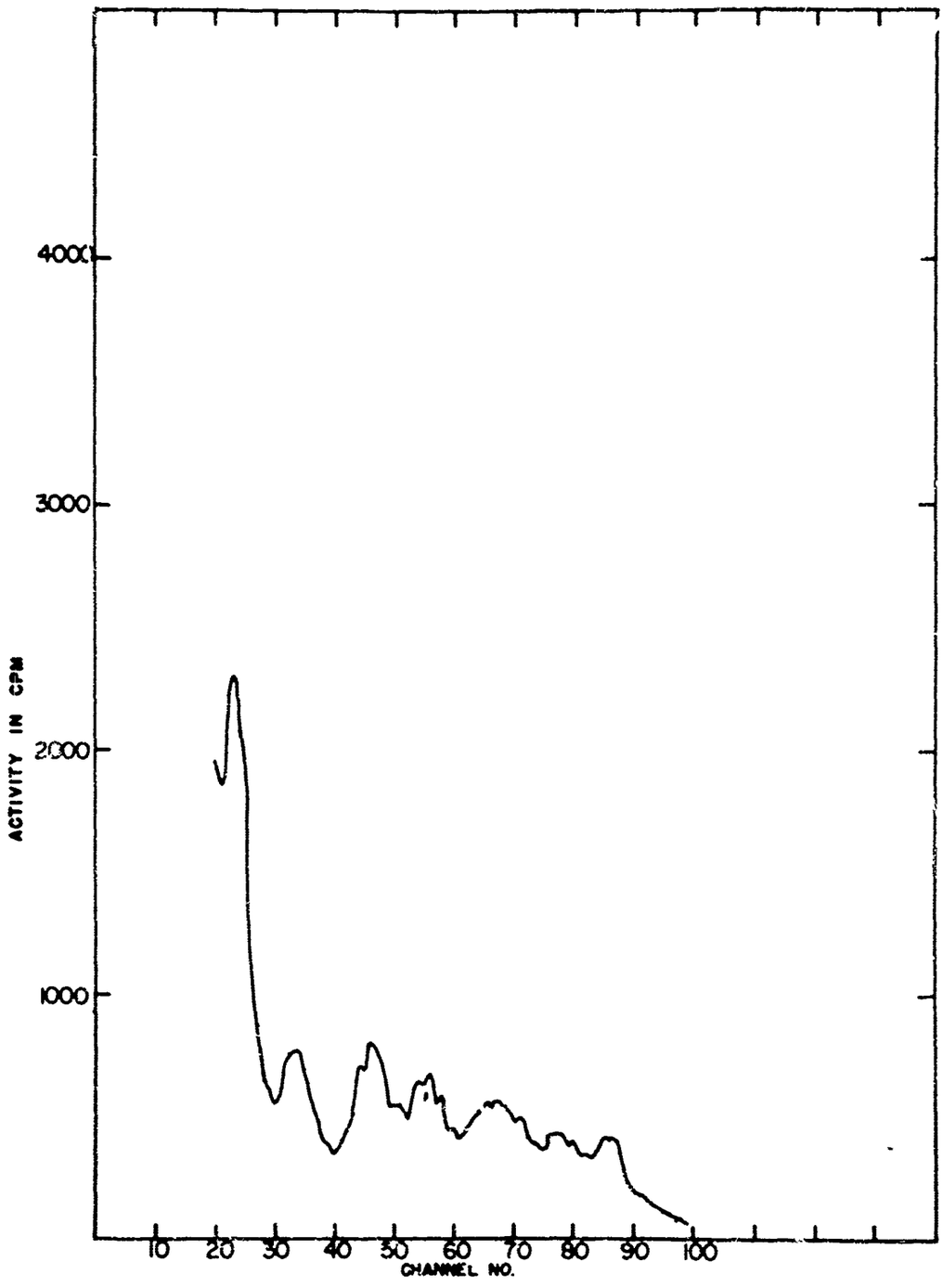

Fig. 12.1—Pteropod sample X-1. One-minute counts per channel; 0430, May 21, 1955.

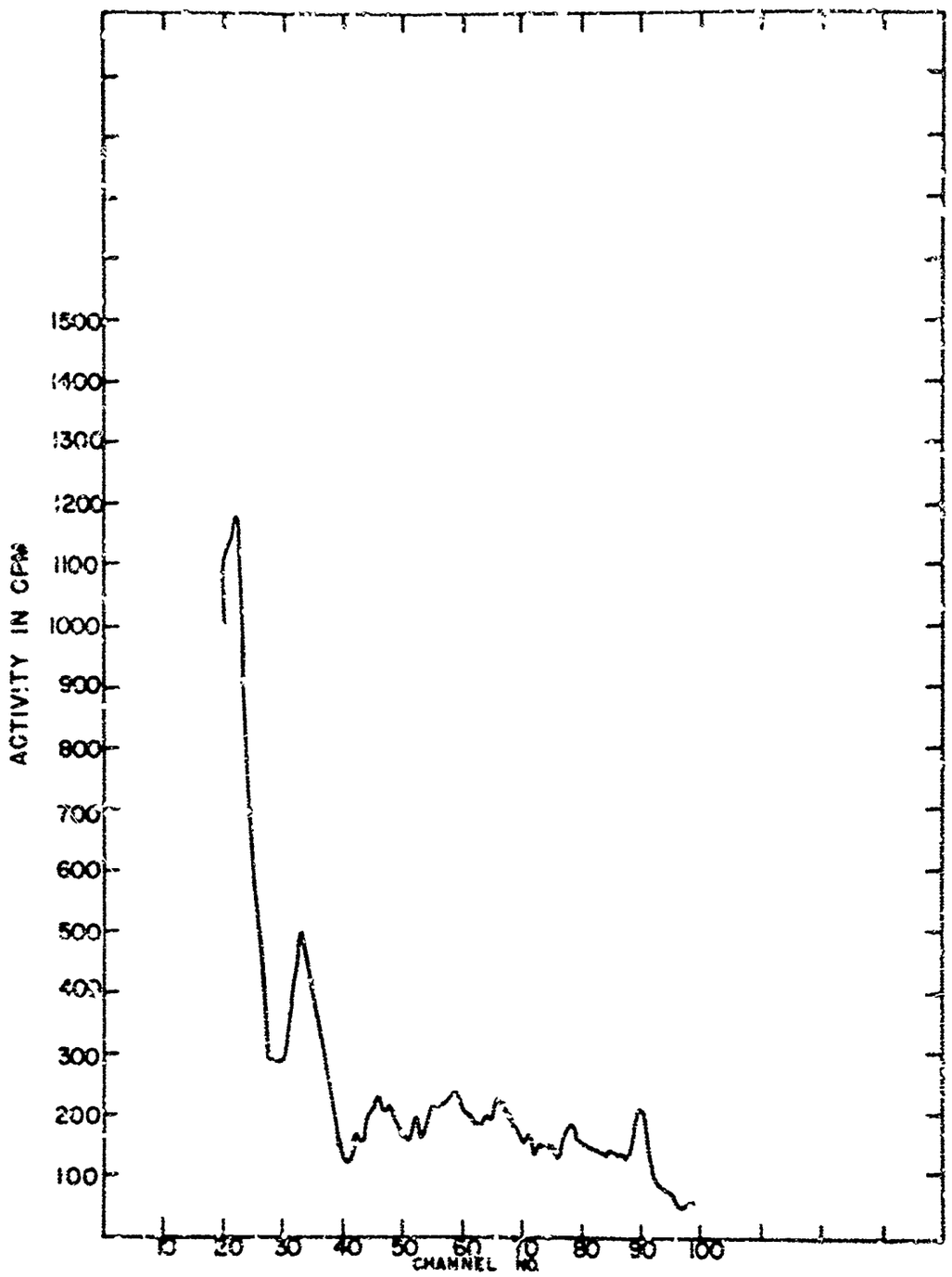

Fig. 12.2—Water sample 4, unfiltered. One-minute counts per channel; 0950, May 21, 1956.

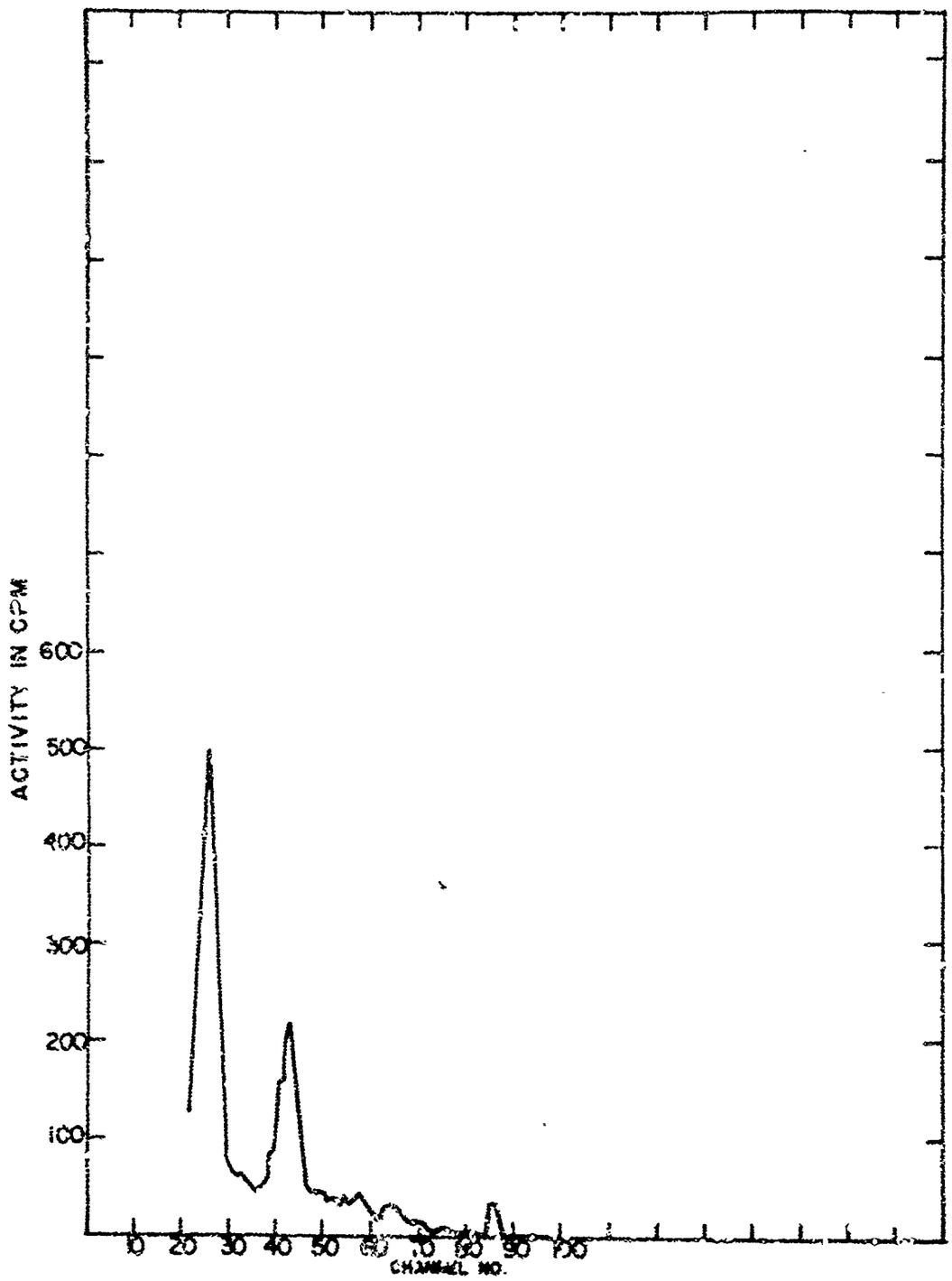

Fig. 12.3—Pteropod sample X-1. Two-minute counts per channel; 0900, June 25, 1965.

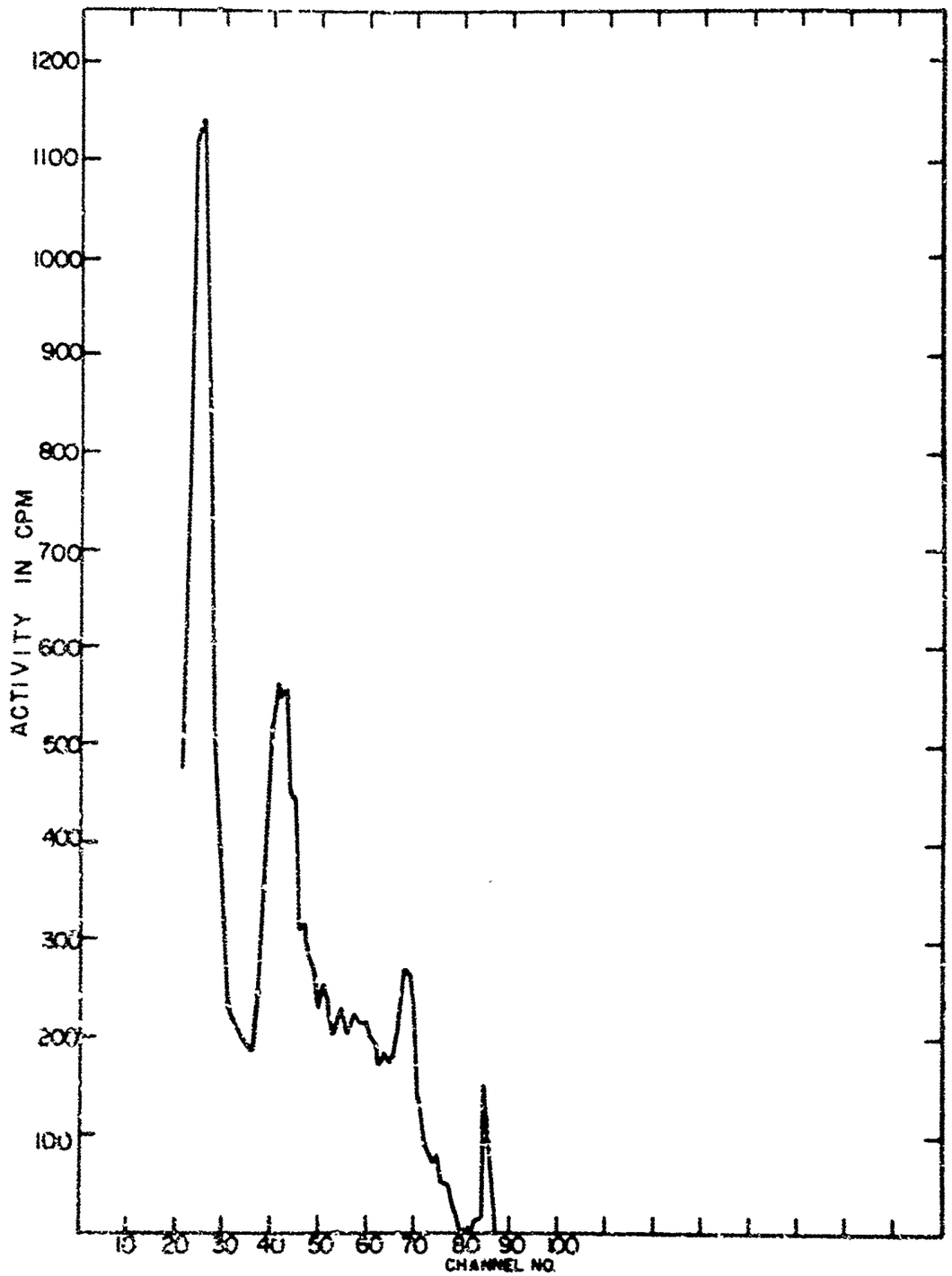

Fig. 12.3—Water sample 4, unfiltered. Seven-minute counts per channel; 1323, June 24, 1955.

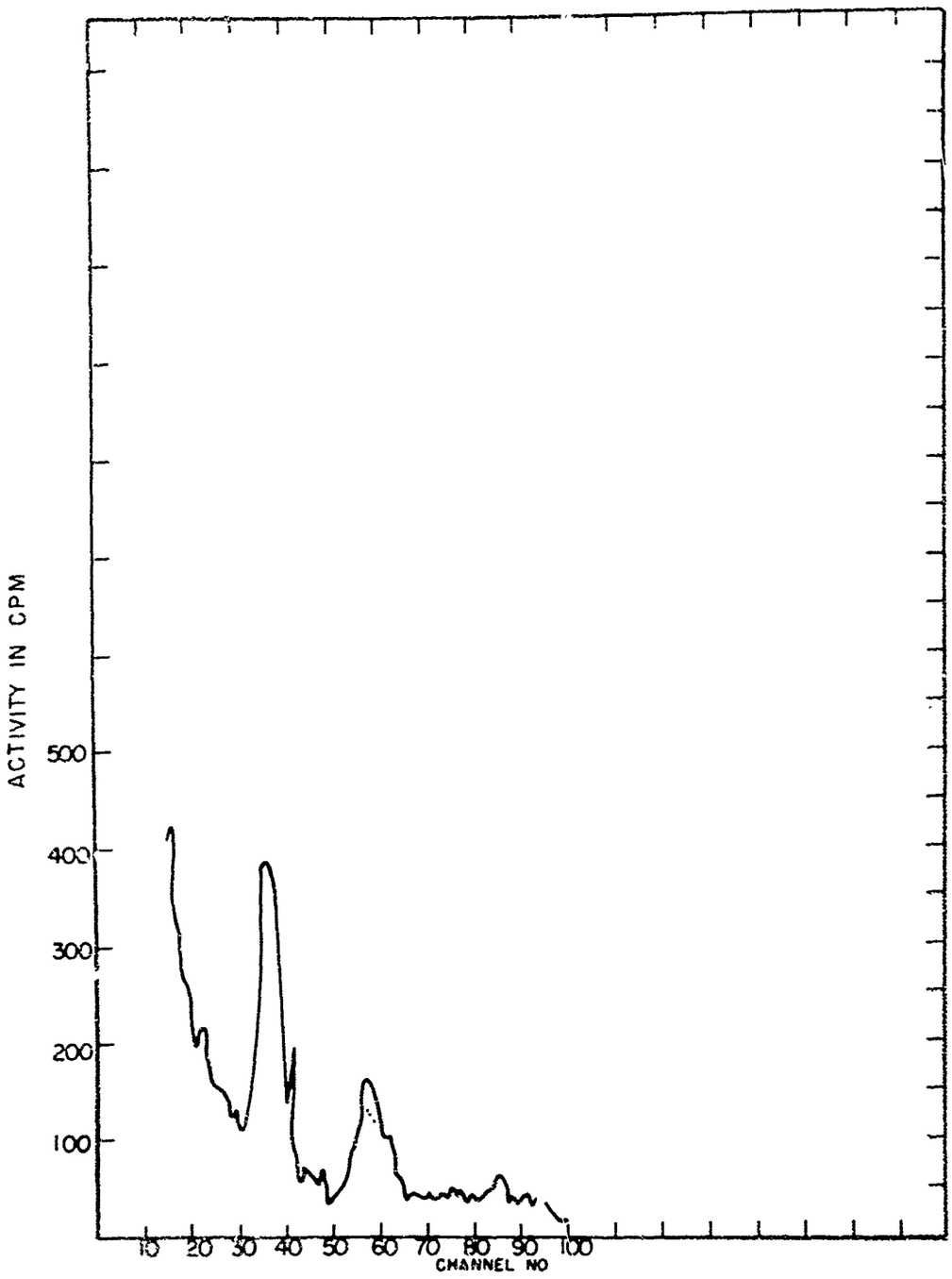

Fig. 12.5—Pteropod sample X-1. Ten-minute counts per channel; 1500, Aug. 1, 1955.

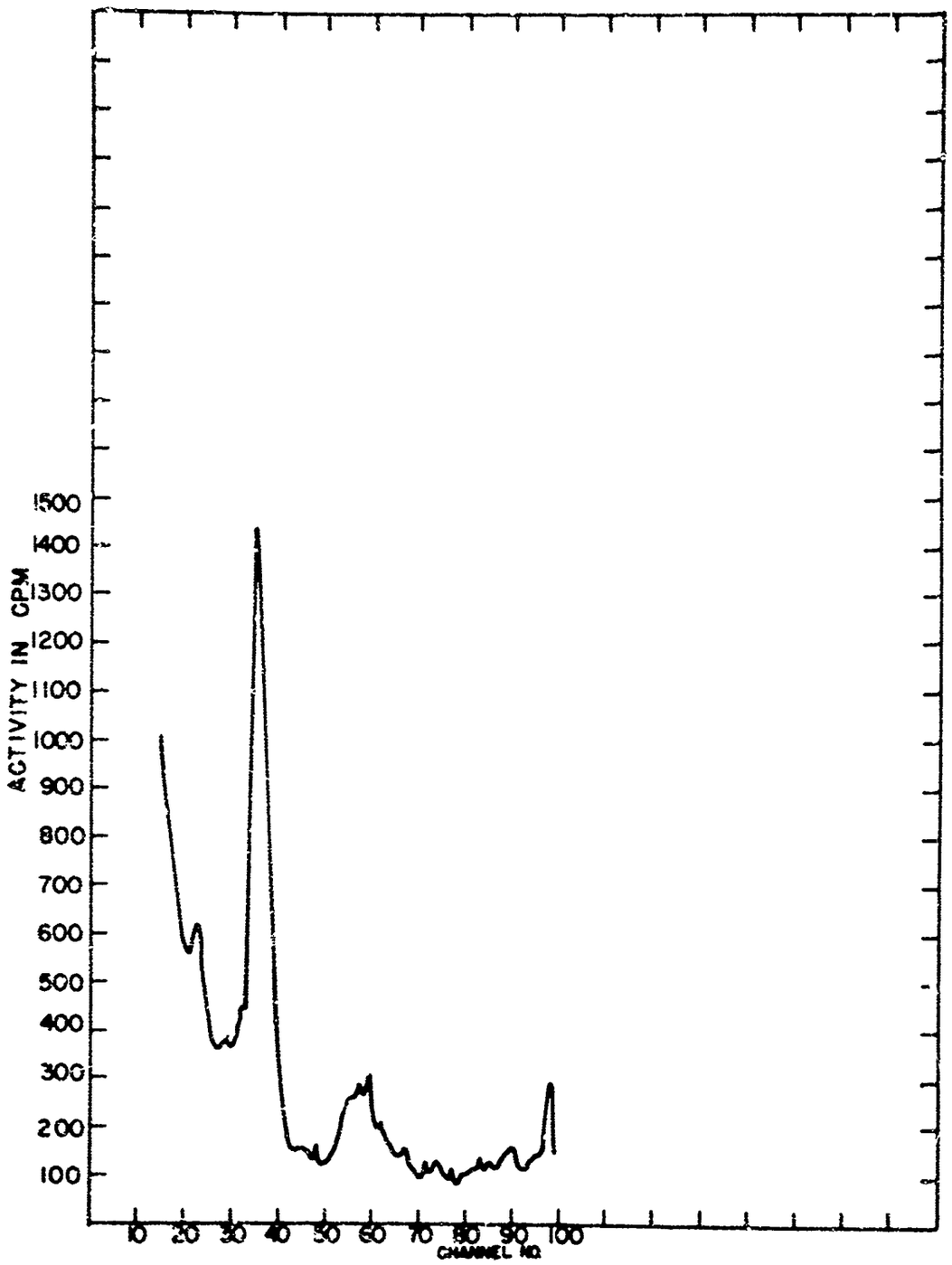

Fig. 12.6—Water sample Folsom No. 5. Ten-minute counts per channel; 0900, July 28, 1955.

Table 12.10—GAMMA ACTIVITY OF ORGANISMS COLLECTED ON BAIRD HAUL 5, MAY 24, 1955
(Samples Counted June 14, 1955)

Sample No.	Feeding Type	Organism	Wet Weight (grams)	Activity	Activity per Gram
E-17	M	Small colonial tunicate	0.54	12,494	23,200
E-9	S	Mysid	1.0	46	46
E-10	S	Pink Shrimp	0.5	5	10
E-11	S	White Shrimp	0.6	30	50
E-14	S	Euphausiid	0.18	16	89
E-15	S	Transparent crustacean, long appendages	0.46	899	1950
E-16	T	Small purple jellyfish	1.65	377	230
E-1	Fish	Lampanyctus idostigma	0.8	7	9
E-2	Fish	Melamphaes bispinosus	3.7	93	25
E-3	Fish	Lampadena sp.	1.8	116	65
E-4	Fish	Ceratoscopelus townsendi	2.3	2402	1040
E-5	Fish	Argyropelecus lychnus	2.65	1087	410
E-6	Fish	Nemichthys scolopaceus	1.8	44	24
E-7	Fish	Lampanyctus mexicanus	2.7	27	10
E-8	Fish	Diaphus sp.	1.3	272	210
E-12	Fish	Danaphos oculatus	0.25	14	56
E-13	Fish	Myctophum sp.	0.24	37	90

Table 12.11—BETA ACTIVITY OF ORGANISMS COLLECTED ON BAIRD HAUL 5, MAY 24, 1955
(Samples Counted June 14, 1955)

Sample No.	Feeding Type	Organism	Wet Weight (grams)	Activity	Activity per Gram
EC-10	M	Colonial tunicate		3	
EOu-7	S	White shrimp		2	
EOu-8	S	Mysid		6	
EOu-9	S	Amphiped		0	
EOu-12	S	Big-eyed shrimp		132	
EOu-13	S	" "		44	
EC-1	Fish	Cyclothone signata		14	
EOu-2	Fish	Cyclothone acclinidens		19	
EOu-3	Fish	Diogenichthys laternatus		1	
EOu-4	Fish	Ceratoscopelus townsendi		4	
EOu-5	Fish	Lampadena sp.		7	
EOu-6	Fish	Lampanyctus idostigma		20	

Table 12.12—BETA ACTIVITY OF BONITO SHARK 5505-P-8, LENGTH 70 CM, CAUGHT MAY 23, 1955

Organ	Wet Weight (Grams)	Beta Activity in CPM	%
Stomach contents (Liquid)	180 g.	0	0
Stomach	74	22	0.3
Intestine	69	20	3.5
Intestine contents	20	8	2.5
Liver	75	14	5.3
Dorsal flesh	148	39	0.33
Heart	8	0	0
Testes	2.5	0	0

Table 12.13—GAMMA ACTIVITY OF BLUE SHARK 5505-P-9, CAUGHT MAY 23, 1955

Organ	Wet Weight (Grams)	Gamma Activity in CPM
Stomach contents	1	19
Gills	39	158
Liver	38	0
Spleen	3.2	0

Table 12.14—GAMMA ACTIVITY OF HATCHET FISH E-5 FROM HAUL 5, MAY 24, 1955

Organ	Activity in CPM
Lower intestine	81
Pyloric caeca	39
Liver	10
Fat deposit	6
Heart	4
Pectoral bone	5
Lower Jaw	11
Gills and Gill arches	35
Eyeball	30
Brain	5
1/4 of skin	4
Flesh	2
Tail fin	11
Pectoral fin	11
Stomach contents	951
Stomach	15
Spleen	5
Tissue surrounding spleen	14

Table 12.15—GAMMA ACTIVITY OF LANTERN FISH FROM HAUL 4, MAY 22, 1965

Organ	Wet Weight (Grams)	Activity in CPM
Gills	55	19
Gill covers	1.2	413
Brain case and eyes	1	21
5 nematode parasites	0.005	0
Ovaries	0.65	23
Heart	0.04	3
Liver	0.25	14
Caudal fin	0.28	4
Pectoral and pelvic fin	0.86	36
Muscle	2.84	56
Backbone	1.32	29
Stomach contents	0.05	14
Stomach	0.5	49
Gut and caeca	0.11	33
Intestines and feces	0.05	13

tory studies of fish collected at random from contaminated water, can result in only generalized statements. Such factors as the interval between ingestion of contaminated food and the time of capture of the specimen, as well as information as to the kind or number of contaminants and the rate of assimilation by the fish, prevent clear and definite conclusions until such factors are subjected to laboratory analyses on living fishes.

Such laboratory studies have indicated that in the Pacific mackerel, Pneumatophorus japonicus diego (Ayres), the final concentration of radioactive elements takes place in the bone structure. At present there is no good estimate of how long, or at what level of activity, these elements may remain in the bone structure. Of immediate concern, regardless of the ultimate focuses and duration of concentration, is the fact that the forage fishes investigated do become contaminated. The various zooplankters found to concentrate activity are all, either directly or indirectly, members of the food chain and are potential food for the forage fishes. Almost all fishes studied for this report may be classified as forage fishes and as food for the larger commercial fishes, primarily the tunas.

12.4 CONCLUSIONS

As a first approximation to the interaction of the marine biosphere and marine hydrosphere in which an atomic detonation has occurred, the following conclusions may be drawn from the preceding data:

1. The most effective concentrators of the activity (which exists mainly in the particulate form) are the mucous, ciliary, and pseudopodial filterers. The members of this group are zooplankton.

2. From the limited assays on the diatoms, apparently the types of phytoplankton in the area were not effective accumulators of activity. This result may have arisen from improper handling (i.e., the protoplasm may have been lost) during washing.

3. No significant concentrations of activity were found in the fish, other than accumulations in the stomach and gut regions. No long-term studies for sites of accumulation of specific isotopes were made.

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